EFFECT OF ENZYMES ON THE DIAUXIC LAG OF DENITRIFYING BACTERIA SWITCHING BETWEEN ELECTRON ACCEPTORS

By

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The hypothesis that presence of the periplasmic nitrate reductase during aerobic growth minimizes or eliminates the diauxic lag was tested. Indeed, little or no lag is observed when bacteria known to express the periplasmic enzyme (*Paracoccus pantotrophus)* are subjected to an anoxic environment after a period of aerobic growth. Long lags were still observed when aerobically grown cultures of bacteria lacking the periplasmic enzyme (*Pseudomonas denitrificans*) were switched to anoxic conditions. There was some indication that more oxidized substrates led to longer diauxic lag lengths for *Paracoccus pantotrophus* although no long lags were observed for any of the substrates with this species. Enzyme assays were performed to confirm activity of the enzyme

The periplasmic nitrate reductase is not expressed in all denitrifying bacteria. However, all denitrifying bacteria have a membrane bound nitrate reductase to catalyze the use of nitrate as electron acceptor. A simple model has been formulated to simulate diauxic growth of denitrifying bacteria that only have the membrane-bound enzyme. It successfully depicts the observed lags when a pure culture of *Pseudomonas denitrificans* switches from oxygen to nitrate as terminal electron acceptor. The model includes the effect of carbon substrate limitation and length of aerobic phase; and does not run into problems when switching from anoxic to aerobic conditions, unlike earlier models of diauxic growth. In order to fit growth data of bacteria that also have the periplasmic nitrate reductase, a preliminary model was devised that includes the contribution of both enzymes.

CHAPTER 1
INTRODUCTION

Removal of nitrogen compounds from wastewater is very important to satisfy increasingly stringent effluent quality requirements. There are several reasons why this is a necessary process. Presence of nitrogen contributes to eutrophication of bodies of water, exerts an oxygen demand that can cause harmful effects on aquatic life and the environment, and is toxic in some forms (Ramalho, 1983). Biological processes are the preferred means of removing nitrogen from wastewater (Grady et al., 1999).

Biological nitrogen removal in wastewater treatment plants is achieved through bacterially mediated oxidation of ammonia to nitrate; and reduction of nitrate to nitrite, nitrous oxide, nitric oxide, or dinitrogen. Accumulation of any of the intermediates (i.e., nitrite, nitric oxide, or nitrous oxide) is undesirable. Biological oxidation of ammonia to nitrate is termed nitrification and is carried out by aerobic, autotrophic bacteria (e.g., *Nitrosomonas* and *Nitrobacter*). Biological reduction of nitrate to dinitrogen is known as complete denitrification. Denitrification is carried out by heterotrophic, facultative anaerobic bacteria (e.g., *Pseudomonas denitrificans*, *Paracoccus pantotrophus*, *Pseudomonas stutzeri*). Although denitrification under aerobic conditions has been reported, the rate of denitrification is maximized under anoxic (dissolved oxygen absent, nitrate present) conditions (Tiedje, 1988). Therefore, to achieve nitrogen removal in a single-sludge system, a mixed population of nitrifying and denitrifying bacteria must be exposed to alternating aerobic and anoxic conditions (Ramalho, 1983). In order for this process to be efficient, the bacteria must be able to adapt quickly to these changes

Several investigators (Gouw et al., 2001, Liu et al., 1998a, 1998b, Lisbon et al., 2002) have shown that when bacteria change from dissolved oxygen to nitrate as electron acceptors, they can experience a period of little or no growth. Such a period, when it occurs between two periods of exponential growth, was termed diauxic lag by Monod (1942). The same terminology is used in this study. Lag periods, since they reduce the overall rate of nitrogen removal, are undesirable in nitrogen removing wastewater treatment processes. It is believed that during this lag bacteria will produce the membrane-bound nitrate reductase, an enzyme that is inhibited by oxygen and is necessary to consume the less-preferred substrate (nitrate).

Some researchers have reported simultaneous utilization of nitrate and oxygen (Robertson and Kuenen, 1984) by *Thiosphaera pantotropha* (now known as *Paracoccus pantotrophus*). These bacteria are known to have a periplasmic nitrate reductase (Nap) that is not inhibited by oxygen. It has been suggested that a possible role for this enzyme is to aid in the transition from environments where oxygen is present to environments where it is absent (Moreno-Vivián et al., 1999). If this is the case, there is a possibility that the diauxic lag due to a switch between oxygen and nitrate as electron acceptor could be minimized or completely eliminated for bacteria containing this enzyme. It has also been suggested that Nap is used for redox balancing and that the activity of Nap increases with the extent of reduction of the carbon substrate used for growth (Richardson and Ferguson, 1992). Thus, it is possible that the diauxic lag length of Nap-containing bacteria will decrease as the reduction state of the carbon substrate increases.

CHAPTER 2
REVIEW OF LITERATURE

Nitrogen Removal

Nitrogen removal can be achieved by several physico-chemical as well as biological processes. Reactions generating nitrogen-containing gases catalyzed by chemical agents are known as chemodenitrification. An example of these is the acid-catalyzed destruction of nitrite, in which the predominant product is usually NO, which is undesirable (Tiedje, 1988). The gas, NO, is a very minor product of biological denitrification, which is one of the reasons why biological denitrification is a very attractive method for nitrogen removal. Furthermore, physico-chemical technologies are expensive and difficult to maintain (Grady et al., 1999).

Biological nitrogen removal from municipal wastewater requires both nitrification and denitrification. In nitrification, ammonia is oxidized to nitrite (NO_2^-) in the presence of oxygen by specific bacteria (*Nitrosomonas*) and, in turn, nitrite is oxidized to nitrate (NO_3^-) in the presence of oxygen by other specific bacteria (*Nitrobacter*). In denitrification, nitrate is converted to nitrogen gases (NO, N_2O, N_2) in the absence of molecular oxygen (Ramalho, 1983).

Many of the bacteria that can denitrify are facultative anaerobes, such as *Pseudomonas denitrificans*. These bacteria can grow under both anoxic and aerobic conditions (Kornaros and Lyberatos, 1998). However, oxygen respiration is more energetically efficient than denitrification (respiration of nitrogen oxides), so facultative bacteria usually utilize nitrogen oxides only in the absence of oxygen (Oh and Silverstein, 1999b).

A number of approaches were taken when developing the basis for biological nitrogen removal. One involved the use of a series of separate suspended growth systems to accomplish removal of organic matter and nitrogen in a step-wise fashion. Organic matter was removed in the first step, nitrification occurred in the second, and denitrification occurred in the third (with addition of an external carbon source). These systems involved high construction and operating costs. Another approach, involving the incorporation of aerobic zones for nitrification and anoxic zones for denitrification in a single system, with carbon oxidation occurring in both zones, was developed. It was referred to as single-sludge nitrogen removal (Grady et al., 1999). In these systems, the bacterial population must be alternately exposed to aerobic and anoxic environments. This forces the biomass to switch between electron acceptors, using oxygen during the aerobic phase and nitrate in the absence of oxygen.

The required sizes of facilities for biological denitrification depend on the kinetics of denitrifying bacteria. Several factors may affect denitrification kinetics. For example, the quantities of nitrogen oxide reductases (enzymes required for utilization of nitrogen oxides) are affected by induction (if present) by NO_3^-, NO_2^-, NO, and N_2O, or repression by O_2. O_2 might decrease enzyme activity due to competition for electrons or by enzyme inactivation. Limited electron donor (shortage of available carbon) might also reduce enzyme activity, as might other factors, such as, pH, temperature, and/or toxic chemicals (Tiedje, 1988).

Regulation of Enzymes Involved in Denitrification

Reduction of nitrate in anaerobic environments occurs mostly by two dissimilatory processes respiratory denitrification and dissimilatory nitrate reduction to ammonium Although assimilatory nitrate reduction could also occur presence of high concentrations of ammonium and organic nitrogen which commonly exist in anaerobic environments, repress this process Dissimilatory processes differ from assimilatory processes by the fact that the reduced nitrogen is not used by the cell Nitrogen oxides in dissimilatory processes serve as electron acceptors for the cell's metabolism (Tiedje, 1988)

Oxygen regulates denitrification by repressing enzyme synthesis or by inhibiting enzyme activity (Tiedje, 1988) It is generally accepted that the respiratory nitrate reductase and other respiratory nitrogen oxide reductases are repressed or inhibited by O_2 to different extents (Tiedje, 1988 Payne 1981) The later reductases (for NO and N_2O) in the denitrification sequence are somewhat more O_2 sensitive than the earlier reductases (Knowles, 1982) Bonin et al (1989) studied the effects of oxygen on each step of denitrification by *Pseudomonas nautica*, and observed that every enzyme associated with denitrification is affected differently with respect to oxygen concentration Nitrate reductase turned out to be less sensitive to oxygen than the other nitrous oxide reductases were At oxygen concentrations greater than 4 05 mg/L, nitrate reductase was completely inactivated while nitrite and nitrous oxide reductase activities were blocked at 2 15 mg/L and 0.25 mg/L respectively McKenney et al (1994) studied the effects of oxygen on the kinetics of denitrification by *Pseudomonas fluorescens*. Their experiments showed that oxygen inhibited all steps in the denitrification process. The inhibition was rapid after introducing oxygen and was reversible after restoring anoxic conditions The transient effects suggested that the reduction of NO_2^-, NO and N_2O are each inhibited to increasing extents by oxygen. However, completely anaerobic conditions were not necessary for denitrification to occur.

The reduction of nitrate to nitrite is catalyzed by three different bacterial enzymes: the cytoplasmic assimilatory (Nas), the membrane-bound respiratory (Nar), and the periplasmic dissimilatory (Nap) nitrate reductases.

Regulation of Nas by ammonium takes place in two ways: repression by the general nitrogen regulatory system (Ntr), which regulates synthesis of most enzymes required for use of alternate nitrogen sources; and inhibition of nitrate transport, avoiding induction of nitrate reductase (Moreno-Vivián et al., 1999).

A membrane-bound nitrate reductase (Nar) has been identified in several nitrate-utilizing bacteria. The activity of Nar in bacteria is inhibited by oxygen. Observations with *Paracoccus denitrificans* indicated that this is not a direct inhibition. Instead, it is exerted via the redox state of a cellular component controlling the movement of nitrate across the cytoplasmic membrane to the active site of the reductase (Alefounder and Ferguson, 1980). Nar is predominantly expressed during growth under anaerobic conditions with nitrate present and is only operative in the absence of oxygen (Bell et al., 1990). However, Krul and Veeningen (1977) found that in *Alcaligenes* strain 15, Nar only partially lost activity in the presence of oxygen. Thus, it is not conclusive that in all microorganisms Nar is inoperative under aerobic conditions. Azide, cyanide, and thiocyanate have been found to inhibit this enzyme (Hochstein and Tomlinson, 1988).

The second dissimilatory nitrate reductase (Nap), which is located in the periplasm, has been found in aerobically denitrifying bacteria. Nap is predominantly expressed when cells are grown aerobically, but can operate in either the presence or absence of oxygen. Nap is not affected by presence of ammonium. Patureau et al. (1994), in their studies of denitrification by *Comamonas* sp. under aerobic conditions, concluded that, under certain conditions, oxygen had a stimulatory effect on the activity of previously synthesized Nap.

Several authors have suggested that Nap is a dissimilatory enzyme used to shunt excess electrons to nitrate for redox balancing (Richardson and Ferguson, 1992, Sears et al., 2000). Redox balancing can be necessary for optimal bacterial growth under some physiological conditions, such as oxidative metabolism of highly reduced carbon substrates in aerobic heterotrophs (Moreno-Vivián et al., 1999). The level of Nap activity during aerobic growth has been shown to increase with the extent of reduction of the carbon substrate in cultures of *Thiosphaera pantotropha*. A possible reason for this is that maintenance of redox balance during aerobic growth on more reduced carbon substrates may be more difficult (Richardson and Ferguson, 1992). The genetic regulation leading to the activity change in response to redox state is still not clear. However, a recent analysis by Richardson and colleagues (2001) revealed the existence of two promoters upstream of the *nap* gene cluster. One promoter is more active during aerobic growth on reduced carbon substrates and the other during aerobic growth on oxidized carbon substrates. The activity of both promoters is repressed under anaerobic conditions, when *nar* genes are expressed.

Because Nap is located in the periplasm, nitrate transport limitation by oxygen (as in the case of the Nar system) is not expected. Aerobic denitrification is then possible, which could be valuable to organisms growing under microaerobic conditions. Bedzyk et al (1999) proposed that Nap is required to catalyze the first step of denitrification in *Pseudomonas* sp Strain G-179 They saw no growth of a Nap-deficient mutant under denitrifying (anoxic) conditions It is not clear under what conditions cultures were grown prior to the anoxic conditions Perhaps there was simply a lag and they did not wait long enough for the cultures to build up Nar and begin anoxic growth.

It has also been suggested that Nap may be utilized in the adaptation to metabolism in the absence of oxygen after transition from aerobic conditions (Moreno-Vivián et al , 1999) This latter suggestion is especially pertinent to wastewater treatment plants that alternate the environment of biological populations between aerobic and anoxic states

Warnecke-Eberz and Friedrich (1993) studied the activities of three nitrate reductases (Nas, Nar, and Nap) in *Alcaligenes eutrophus* strain H16 In agreement with other studies, they found that oxygen shuts down synthesis of Nar and that ammonium shuts down synthesis of Nas Interestingly, the activity of Nap in this organism was only observed during stationary phase whereas Richardson and Ferguson (1992) found that, in *Thiosphaera pantotropha* the periplasmic dissimilatory nitrate reductase was expressed during exponential growth.

The dissimilatory nitrate reductase of most microorganisms is induced by nitrate under anaerobic conditions. However, sometimes nitrate does not need to be present to induce this enzyme (Schulp and Stouthamer, 1970; Krul and Veeningen, 1977)

It is commonly believed that oxygen represses the synthesis of the membrane-bound dissimilatory nitrate reductase (e.g., Warnecke-Eberz and Friedrich, 1993, Moreno-Vivian et al , 1999) Krul and Veeningen (1977) studied the synthesis of nitrate reductase under aerobic conditions by various denitrifying bacteria isolated from activated sludge and drinking water, including *Alcaligenes* strains 15 and N4. Although chlorate can be reduced by the dissimilatory nitrate reductase (Pichinoty, 1965), it was shown to repress the synthesis of the dissimilatory nitrate reductase in strain 15. The nitrate reductase activity measured with cells precultured in the presence of high amounts of chlorate is comparable to the activity measured in cells grown at high dissolved oxygen concentrations without chlorate. This can be explained by the hypothesis of Wimpenny and Cole (1967) that redox potential is the regulating factor for synthesis of dissimilatory nitrate reductase. Thus, the redox potential-increasing effect of chlorate might be responsible for the repression. However, synthesis of dissimilatory nitrate reductase was not seriously repressed by chlorate in strain N4, suggesting either a different regulation pattern, or the possibility that the dissimilatory nitrate reductase in N4 is constitutive. It seems that, at least for some bacteria, it is redox potential, as opposed to oxygen concentration per se, that represses biosynthesis of membrane-bound dissimilatory nitrate reductase.

Effect of Oxygen on Nitrogen Removal through Denitrification

As the previous discussion on enzyme regulation makes clear, oxygen serves as an inhibitor of activity or as a repressor of synthesis of denitrification enzymes. The existence of a threshold oxygen concentration above which denitrification cannot occur has been suggested because of the observed effect of oxygen on nitrogen removal through denitrification. This implies a critical concentration instead of a gradual inhibitory response. Different values have been reported and show that active denitrifying microorganisms in marine, freshwater, and soil environments have very low thresholds for oxygen. However, wastewater environments that provide an excess of electron donor and a fluctuating status of nitrate and oxygen might select for organisms that could make simultaneous use of both nitrate and oxygen as electron acceptors (Tiedje, 1988). This phenomenon has been termed aerobic denitrification

Aerobic denitrification has been observed by several authors Robertson and Kuenen found that *Thiosphaera pantotropha* (LMD 82 5) is capable of simultaneously utilizing nitrate and oxygen as terminal electron acceptors in respiration This behavior has been found in cultures maintained at dissolved oxygen concentrations as high as 90% of air saturation (Robertson and Kuenen, 1984). When using both terminal electron acceptors simultaneously, batch cultures of *Thiosphaera pantotropha* grew more rapidly than when supplied with either electron acceptor alone. In two-member chemostat cultures containing *Thiosphaera pantotropha* and *Paracoccus denitrificans* (LMD 22 21) a strictly anaerobic denitrifier the first was relatively unaffected by aerobic/anaerobic switches, as long as nitrate was present. However the latter washed out when suddenly switched from aerobic to anaerobic conditions, possibly because it was unable to synthesize its denitrification enzymes quickly enough (Robertson and Kuenen, 1990)

Strain SGLY2 of *Comamonas* sp. also is capable of denitrifying under aerobic conditions (Patureau et al 1994) According to their study oxygen was used simultaneously with nitrate for respiration, giving a growth rate ten times greater than that obtained when only nitrate was used as an electron acceptor Nitrogen gas production was observed during aerobic nitrate reduction.

Denitrification at positive bulk dissolved oxygen concentrations has been widely observed in activated sludge (e g Krul 1976 O'Neill and Horan, 1995 Munch et al , 1996, Goronszy, 1997). The typical observations, as reiterated by Oh and Silverstein

(1999a) are that low dissolved oxygen concentrations decrease the specific denitrification rate and this effect is amplified with increasing dissolved oxygen concentrations. Furthermore, high dissolved oxygen concentrations produce almost complete inhibition of denitrification. At dissolved oxygen concentrations as high as 2 mg/L, they found that denitrification continued, although at a greatly reduced rate.

Several possible explanations exist for this behavior. One is the presence of aerobically denitrifying bacteria, such as *Thiosphaera pantotropha* and *Comamonas* sp strain SGLY2, as described above. It could also be that, even if oxygen inhibits the denitrifying activity of bacteria, anoxic regions can exist in an activated sludge floc due to limitation of oxygen diffusion within the floc. These micro-niches allow nitrification to occur in the outer region in contact with bulk water DO (dissolved oxygen), and denitrification in an inner anoxic zone.

Krul (1976) did a study to compare the oxygen uptake and dissimilatory nitrate reduction by cells in suspension and cells in flocs. A number of bacteria were isolated from aerobically grown and denitrifying activated sludge and were tested for their capacity to form flocs, sensitivity of the flocs to dispersion, and ability to denitrify. Flocs of anaerobically grown cells of *Alcaligenes* strain 15 showed greatly increased nitrate reduction below 1.5 mg/L oxygen as opposed to cells in suspension, which did not show increased reduction until the concentration of oxygen was close to zero. This difference in behavior became more evident when flocs and suspended cells had been pre-aerated in a nitrogen free medium for 24 hours before being tested.

Thus, the anaerobic, denitrifying inner part of flocs may be responsible for the loss of nitrogen in activated sludge plants under aerobic conditions. This explanation has sometimes been termed Simultaneous Nitrification/Denitrification (SND). When studying the factors affecting SND, it was shown that increased dissolved oxygen concentrations negatively affect SND, and that SND activities increased in reactors with larger activated sludge floc sizes (Pochana and Keller, 1999).

Kugelman et al. (1991) gave a third explanation for aerobic denitrification. They found that nitrite had an inhibitory effect on oxygen respiration. In their studies, the inhibitory effect persisted at nitrite levels as low as 1 mg/L and was observed in activated sludge grown with an alternating anaerobic/aerobic sequence. Because of this inhibition, the biomass continued to use nitrogen oxides as electron acceptors, even at high DO concentration.

Zhao et al. (1999) rejected Kugelman and colleagues' (1991) explanation for aerobic denitrification, since the nitrite concentrations in their systems were very low (less than 0.15 mg N/L) and thus would not exert a significant inhibitory effect on oxygen respiration. The theory of the anoxic microzone inside the flocs did fit some observations in their study. When investigating the effect of intermittent aeration on their systems their results showed that a longer aeration cycle (with a zero-DO period) promoted Sequential Nitrification/Denitrification (SQND) (i.e., anoxic denitrification and autotrophic nitrification) (Zhao et al., 1999).

Effect of Cycling Air On and Off

Kawakami et al. (1985) examined inhibition of denitrification starting from nitrite (denitritification) by oxygen in *Paracoccus denitrificans* by alternating aerobic and anaerobic conditions in batch reactors. The cells were grown anaerobically with added $NaNO_2$ and then aliquots of the cell suspension were exposed to different oxygen concentrations. The inhibition of nitrous oxide evolution increased as the dissolved oxygen concentration increased. The fully aerobic sample did not release nitrous oxide. When switching from aerobic conditions back to anaerobic conditions, the activity of nitrite reductase was recovered. An experiment was performed with two cycles of anaerobic/aerobic switches. Although inactivation of nitrite reductase occurred in both cycles, the production of nitrous oxide was greater in the second cycle. This suggests that inactivation by oxygen of nitrite reductase synthesized during anaerobic preculture is reversible. Activity of the enzyme is lost under aerobic conditions, and is regained upon switching to anaerobic conditions. To prove that the nitrite reductase from the initial anaerobic phase was responsible for the nitrite reducing activity in the second anaerobic phase, chloramphenicol was added just before shifting from aerobic to anaerobic conditions. This would halt all protein synthesis in the ensuing anaerobic period. The evolution of nitrous oxide in the second anaerobic phase was not affected. The persistence of nitrite reductase was examined for various durations of aerobic periods. The remaining activity (measured under anaerobic conditions) was practically the same after up to 30 hours of exposure to aerobic conditions (Kawakami et al., 1985).

Otte et al. (1996) studied denitrification (nitrite reduction) by *Alcaligenes faecalis* in continuous reactors operating at different oxygen concentrations or switching between aerobic and anaerobic conditions. Nitrous oxide (N_2O) was produced under all conditions, from fully anaerobic to fully aerobic. Nitrite reductase activities under fully anaerobic conditions were much higher than under low dissolved oxygen conditions, indicating higher nitrite consumption under fully anaerobic conditions. Yet, the highest relative N_2O emissions were observed under low dissolved oxygen concentrations. During the transition from anaerobic to aerobic conditions, N_2O immediately started to accumulate and N_2 production decreased. These observations suggest that nitrous oxide reductase decreases faster than nitrite reductase activity with respect to dissolved oxygen, indicating that nitrous oxide reductase is more sensitive to oxygen than nitrite reductase is. When changing from aerobic to anaerobic conditions, nitrite reductase seemed to be present, since N_2O was produced immediately after the switch. However, the activity was not enough to sustain growth until about 20 hours later. After about 27 hours, the nitrous oxide activity became detectable. This suggests that nitrite reductase may be constitutively present in small amounts. It was concluded that under anaerobic conditions the enzymes of denitrification are sequentially induced. When switching from low DO conditions to anaerobic conditions, it took much less time for the nitrite and nitrous oxide reductases to reach their maximum activities, suggesting that at low DO the reductases are induced.

Waki et al. (1980) studied the transient characteristics of *Paracoccus denitrificans* with changes between aerobic and anoxic conditions in batch and continuous cultures. They found that the growth yield coefficients and maximum specific growth rates were lower during anoxic conditions than during aerobic conditions. On the transition from anoxic to aerobic conditions, the nitrate reductase activity was inhibited by oxygen, and the reduction of nitrate stopped. When switching from aerobic to anoxic conditions, carbon source (glucose) consumption and bacterial growth stopped for a few hours, after which nitrate reductase activity started to increase. They observed rapid reduction of nitrate and accumulation of nitrite immediately after the switch in conditions. Nitrite reductase activity started to increase after a high (130 mg/L as N) concentration of nitrite had accumulated.

Baumann et al. (1997) studied the effect of repeated alternating aerobic/anoxic growth conditions on the development of stable denitrification activity (i.e., nitrogen gas was the only product released) with *Paracoccus denitrificans* and with activated sludge in continuous reactors. They found that the length of the anaerobic period influenced biosynthesis of the denitrification enzymes. Long anaerobic periods led to stable denitrification activity producing only dinitrogen. Short anaerobic periods led to nitrite accumulation in the medium. It was concluded that the different denitrification enzymes in these cultures are not induced simultaneously, but sequentially. They found similar results when studying the effects on activated sludge. Additionally, they found that once the denitrification enzymes are synthesized, they are not degraded during aerobic growth conditions. Their catalytic activity is inhibited.

The effect of alternating aerobic and anoxic conditions on denitrification by activated sludge was also investigated by Oh and Silverstein (1999b). After several hours of anoxic denitrification, mixed liquor was aerated for variable amounts of time and then returned to anoxic conditions. The specific denitrification rate in the second anoxic phase was decreased in every case. In order to separate the effects of dissolved oxygen alone from the effects of aerobic growth on denitrification, these investigators performed experiments where substrate was not added during the aerobic phases. Aeration without carbon for long periods of time had no effect on the anoxic specific denitrification rate.

Phenomenon of Diauxie

As mentioned previously, oxygen has two possible modes of action in regulating denitrification: repression of synthesis of nitrogen oxide reductases and inhibition of reductase activity. Significant lags when switching from aeration to anoxic denitrification are likely to be associated with repression, whereas inhibition of enzyme activity may be reversed quickly (Rittman and Langeland, 1985) and therefore should not lead to long lags.

Monod (1942, p. 390) first described the phenomenon of diauxie as being "characterized by a double growth cycle consisting of two exponential phases separated by a phase during which the growth passes through a minimum, even becoming negative in some cases." He found it to occur in media where the organic source was limiting and consisted of mixtures of two carbohydrates. According to Monod (1942, p.391), "each cycle corresponds to the exclusive utilization of one of the constituents of the mixture, due to an inhibitory effect of one of the compounds on the formation of the enzyme attacking the other." The lag period corresponds to the time necessary for the formation of a specific enzyme under the influence of its substrate.

Some of the enzymes encoded on a microbial genome are constantly expressed (termed constitutive), whereas most are only expressed when needed for growth or survival (termed inducible) (review by Egli, 1995). Syntheses of a variety of unrelated, primarily catabolic enzymes (used for the breakdown of organic substrates) are blocked when cells are grown in a medium containing a preferred energy source (Madigan et al., 2000). This is referred to as catabolite repression and can lead to diauxic or biphasic growth if two energy sources are simultaneously present in the medium and if the enzyme needed for the utilization of one of these two substrates is subject to catabolite repression.

A common example of the diauxie phenomenon is growth on a mixture of glucose and lactose. β-galactosidase, the enzyme responsible for utilization of lactose, is inducible (meaning that it is synthesized only when lactose is present). Its synthesis, however, is subject to catabolite repression. Therefore, as long as glucose is present in the medium, synthesis of β galactosidase will not occur. The organism will grow only on glucose, leaving lactose intact. After exhausting the glucose, catabolite repression is no longer in effect, allowing synthesis of β-galactosidase. After a while (when sufficient β-galactosidase has been synthesized) growth on lactose begins. The time required for accumulation of β-galactosidase corresponds to the diauxic lag.

Enzymes subject to catabolite repression require binding of the catabolite activator protein (CAP) to DNA, before RNA polymerase can bind and transcription can take place. The CAP protein binds to DNA only after binding cyclic adenosine monophosphate (cyclic AMP). Glucose inhibits the synthesis of cyclic AMP and stimulates its transport out of the cell, lowering the level of cyclic AMP and preventing binding of RNA polymerase to the promoter.

Under substrate excess conditions in batch cultures, sequential utilization of mixtures of substrates resulting in a diauxic growth pattern is most commonly observed. However, the simultaneous use of different carbon sources has also been found. "The preferential growth pattern (sequential utilization) occurs because synthesis of the enzymes that catalyze the transport/catabolism of the less preferred substrate is completely suppressed in the presence of the preferred substrate. On the other hand, simultaneous utilization of the substrates occurs when suppression of enzyme synthesis is incomplete. Thus, the preferential growth pattern occurs because the enzyme catalyzing the uptake of the less preferred substrate becomes extinct, the simultaneous growth pattern occurs because the enzymes for both substrates coexist." (Narang, 1998). An analogy can be drawn between the sequential versus simultaneous utilization of electron donors (carbon substrates) and the sequential versus simultaneous utilization of electron acceptors (e.g., oxygen, nitrate, nitrite). (Reminder: Aerobic denitrification is the simultaneous utilization of oxygen and nitrate.)

Biphasic growth due to a change in electron acceptors (nitrate to nitrite) was first studied by Kodama et al. (1969). They observed that growth of *Pseudomonas stutzeri* in the presence of 20 mM NO_3 (280 mg/L as N) or higher (with oxygen absent) results in two exponential phases separated by an intermediate phase in which the growth is very low. In the first exponential phase, nitrate is reduced to nitrite, and in the second, nitrite is reduced to nitrogen gas. They explain that "the mechanism behind this behavior is one in which nitrate inhibits nitrite reduction in two ways: by repressing formation of the nitrite-reducing system and by competing with nitrite as an electron acceptor in the oxidative function of cells" (Kodama et al., 1969, p. 864).

Another observation of diauxic lag resulting from a transition from nitrate to nitrite as electron acceptor was reported by Kornaros et al. (1996). They conducted batch experiments in which *Pseudomonas denitrificans* were first grown in the presence of nitrite, with oxygen and nitrate absent. Nitrate was then injected, to give a medium concentration of 33 mg/L as N. Immediately after the injection, nitrite started accumulating, and after nitrate was exhausted, a short lag phase was observed, after which growth resumed on nitrite.

The first observation of a diauxic lag experienced by bacteria switching from oxygen to nitrate as a terminal electron acceptor was reported by Waki et al. (1980). Although they did not use the terms biphasic growth or diauxic lag when discussing their results, the bacterial cultures studied clearly exhibited this phenomenon.

Liu et al. (1998a) observed a diauxic lag when activated sludge switched from an aerobic to an anoxic environment. They later found diauxie when *Pseudomonas denitrificans* (ATCC 13867), denitrifying bacteria that were originally isolated from wastewater, switched between oxygen and nitrate as terminal electron acceptor (Liu et al. 1998b). The length of the aerobic growth phase was found to significantly affect the length of the diauxic lag. Longer lags resulted from longer aerobic growth phases. Contrary to the rapid reduction of nitrate observed by Waki et al. (1980) immediately after *Paracoccus denitrificans* switched from aerobic to anoxic conditions, nitrate consumption by *Pseudomonas denitrificans* was very low during the lag period, and then increased rapidly as exponential growth began to take place. Presence or absence of nitrate during the aerobic phase had a marginal effect on lag length (Liu et al. 1998b).

The influence of preculture conditions on the diauxic lag of *Pseudomonas denitrificans* switching between oxygen and nitrate as terminal electron acceptors was studied by Gouw et al. (2001). (Here, preculture refers to a phase of growth before the start of sequential aerobic and anoxic growth phases.) When bacteria were precultured under oxic conditions with nitrate absent, diauxic lags were longer than when bacteria were precultured in the presence of both oxygen and nitrate. Presence of nitrate during long aerobic phases somewhat compensated for the absence of nitrate during preculture phase. In experiments with anoxic preculture, short lags were usually observed when nitrate was absent from the aerobic phase. Even shorter lags, or no lags at all, were observed when nitrate was present during the aerobic phase.

Kornaros and Lyberatos (1998) found no lag when *Pseudomonas denitrificans* growing with air was switched to anoxic conditions. From conversations with the authors, it became known that the conditions in their experiments were similar to those under which Gouw et al. (2001) found no lags upon the switch.

Lisbon et al. (2002) studied the effect of dissolved oxygen concentration in the aerobic phase on the ensuing lag of *Pseudomonas denitrificans* when switching to anoxic conditions. Exposure of the bacteria to low dissolved oxygen concentrations during the aerobic phase resulted in significantly shorter lags than did exposure to higher concentrations approaching air saturation. The cultures grown under low dissolved oxygen concentrations generally grew faster during the subsequent anoxic phase.

Casasus-Zambrana (2002) studied the effects of dissolved oxygen and aerobic growth on the ensuing diauxic lag and anoxic specific growth rate of *Pseudomonas denitrificans*. A lag was more often observed when the bacteria are subjected to an anoxic environment after a period of aerobic growth than after a period of exposure to air without growth. Presence or absence of substrate during the aerobic phase had no effect on the specific growth rate in the ensuing anoxic phase.

Modeling

In order to optimize the design of wastewater treatment facilities, reliable models must be formulated. Successful models using a cybernetic approach have been developed to simulate the diauxic lag experienced by bacteria switching between electron donors (e.g., Ramkrishna, 1982; Ramkrishna et al., 1984; Kompala et al., 1986). These models propose that bacteria regulate enzymes in a way that will maximize their instantaneous specific growth rate. Narang et al. (1997a) studied whether or not these models could predict behaviors in systems with simultaneous utilization of substrates, in which no diauxic lag takes place. They found that the model predicts preferential utilization patterns for all initial conditions.

Traditional models of single-sludge processes, such as the International Water Association (IWA) Activated Sludge Models (ASM) 1, 2, 2d, and 3 (Henze et al., 2000) do not predict lags when bacteria switch between electron acceptors. Liu et al. (1998a) used the cybernetic approach of Kompala et al. (1986) to modify ASM No. 1 so that it would portray a diauxic lag when activated sludge is switched from aerobic to anoxic conditions. The model they obtained was later modified to show the considerably longer lags observed when *Pseudomonas denitrificans* switched between oxygen and nitrate as electron acceptors, as well as the dependence of this lag on the length of the aerobic growth phase (Liu et al., 1998b). However, it runs into trouble when growth switches from anoxic to aerobic conditions, and does not include the effect of substrate limitation

CHAPTER 3
OBJECTIVES

As the above review of the literature indicates, models previously used to represent diauxic growth of denitrifying bacteria have room for improvement. With this in mind, one of the objectives of this study was to modify the most recent model formulated by a former member of our research group (Liu et al., 1998b). The new model can handle transitions from aerobic to anoxic, as well as, from anoxic to aerobic conditions, and includes the effect of substrate limitation. Experimental data was fit to verify the success of the model. This model only includes the effect of the membrane-bound nitrate reductase on the diauxic lag.

Because the periplasmic nitrate reductase is unaffected by oxygen and one of its proposed roles is to aid in the adaptation to anaerobic metabolism after a transition from aerobic growth (Moreno-Vivián et al., 1999), the question of whether presence of Nap in a culture grown aerobically will minimize or even eliminate the diauxic lag upon a switch to anoxic conditions arises. Furthermore, since the level of Nap activity during aerobic growth has been shown to increase with the extent of reduction of the carbon substrate in cultures of *Thiosphaera pantotropha* (Richardson and Ferguson, 1992), perhaps the diauxic lag length will decrease as the activity of Nap increases.

Therefore, a second objective of this study was to determine the effects of presence or absence of Nap on the diauxic lag. The effect of carbon substrate on length of lag was also examined. Results of growth experiments were used to formulate a preliminary model, which incorporates the effect of Nap.

CHAPTER 4
A SIMPLE MODEL

Model Expressions

The model presented by Lau et al. (1998b) runs into trouble when growth switches from anoxic to aerobic conditions and does not include the effect of substrate limitation. It was therefore modified and this chapter will describe the improved model and show that it can fit the experimental results.

The processes included in the present model are aerobic growth of heterotrophic biomass, anoxic growth of heterotrophic biomass, biomass decay, rate of synthesis of oxygenase (enzyme associated with aerobic growth), rate of synthesis of nitrate reductase (enzyme associated with anoxic growth), and decay of oxygenase and reductase. The components included in this model are biomass concentration (X_b), dissolved oxygen concentration (S_O), nitrate-nitrogen concentration (S_{NO}), carbon substrate concentration (S_S), oxygenase concentration (E_O), and reductase concentration (E_{NO}).

The process rate for anoxic growth is structured similarly to the IWA Activated Sludge Models and is given by

$$\left.\frac{dX_b}{dt}\right|_{anoxic} = \hat{\mu}_{NO} f\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)\left(\frac{S_S}{K_{S,NO}+S_S}\right)\left(\frac{K_I}{K_I+S_S}\right)X_b \qquad (4\text{-}1)$$

where $f = \left(\frac{e_{NO}}{e_{NO,\max}}\right)^{n_{NO}}$, $\hat{\mu}_{NO}$ is the anoxic maximum specific growth rate, e_{NO} the reductase specific level (E_{NO}/X_b), $e_{NO,\max}$ the reductase maximum specific level, n_{NO} the reductase activation response parameter, S_{NO} the nitrate-nitrogen concentration, K_{NO} the nitrate half-saturation coefficient, S_S the soluble substrate concentration, $K_{S,NO}$ the anoxic substrate half-saturation coefficient, K_I the inactivation coefficient, and S_O the dissolved oxygen concentration

The above power law function f portrays the enzyme dependence of the anoxic growth rate. This function should be zero (no anoxic growth) when $e_{NO} = 0$ and one when $e_{NO} = e_{NO\ max}$. As in previous models growth rate is a nonlinear function of the controlling enzyme. The model of Liu et al. (1998b) uses $f = v_{NO}\left(\frac{e_{NO}}{e_{NO\ max}}\right)$, where v_{NO} is a logistic function with two tunable parameters. The proposed power law f fits experimental data at least as well, is considerably simpler, and has only one tunable parameter.

Another modification in the model of Liu et al. (1998b) is the addition of a Monod term to account for carbon substrate limitation. The final modification is the addition of the term $\frac{K_I}{K_I + S_O}$. This term accounts for the fact that oxygen is known to minimize the rate of denitrification (Tiedje, 1988). Because the model of Liu et al. (1998b) did not include this inhibitory effect, it predicted continuation of anoxic growth after a culture switched from anoxic to aerobic conditions.

Analogous to the expression for anoxic growth the expression for aerobic growth is given by

$$\left.\frac{dX_b}{dt}\right|_{aerobic} = \hat{\mu}_O\left(\frac{e_O}{e_{O\ max}}\right)^{n_O}\left(\frac{S_O}{K_{O,H} + S_O}\right)\left(\frac{S_S}{K_{S,O} + S_S}\right)X_b \qquad (4\text{-}2)$$

where $\hat{\mu}_O$ is the aerobic maximum specific growth rate, e_O the oxygenase specific level (E_O/X_b), $e_{O,max}$ the oxygenase maximum specific level, n_O the oxygenase activation response parameter, S_O the dissolved oxygen concentration, and $K_{O,H}$ the oxygen half saturation coefficient

The rate of synthesis of reductase in the model is given by

$$\left.\frac{dE_{NO}}{dt}\right|_{synthesis} = \left(\alpha_{NO1} + \alpha_{NO2}\frac{e_{NO}}{e_{NO,max}}\right)\left(\frac{S_{NO}}{K_{NO} + S_{NO}}\right)\left(\frac{S_S}{K_{S,NO} + S_S}\right)\left(\frac{K_I}{K_I + S_O}\right)X_b \quad (4\text{-}3)$$

where α_{NO1} and α_{NO2} are parameters controlling reductase synthesis rate. The dependence of nitrate reductase synthesis rate on the specific level of the enzyme is reasonable if a product of the enzyme-catalyzed reaction acts as inducer for biosynthesis of the enzyme (Narang, 1997b). Because oxygen is known to have an inhibitory effect on the synthesis of the main (membrane-bound) nitrate reductase (Krul and Veeningen, 1977), a Monod type oxygen inhibition term is included in the nitrate reductase synthesis rate. The model of Liu et al. (1998b) included a variable, u_{NO} in the reductase synthesis rate expression to control enzyme induction. The expression for u_{NO} was complicated and the inclusion of this variable resulted in depression of aerobic growth when switching from anoxic to aerobic conditions. In the present model, the oxygen inhibition term is enough to control reductase synthesis.

Analogously, the rate of synthesis of oxygenase is given by

$$\left.\frac{dE_O}{dt}\right|_{synthesis} = \left(\alpha_{O1} + \alpha_{O2}\frac{e_O}{e_{O,max}}\right)\left(\frac{S_O}{K_{O,H} + S_O}\right)\left(\frac{S_S}{K_{S,O} + S_S}\right)X_b \quad (4\text{-}4)$$

where α_{O1} and α_{O2} are parameters controlling oxygenase synthesis rate. The model of Liu et al. (1998b) included a variable, u_O in the oxygenase synthesis rate, analogous to u_{NO}.

This variable is eliminated, since the presence or absence of nitrate does not affect the synthesis of oxygenase.

The biomass decay rate is given as in the IWA Activated Sludge Models by Equation 4-5

$$\left.\frac{dX_b}{dt}\right|_{decay} = b\,X_b \tag{4-5}$$

Here b is the biomass specific decay rate. The rate of decay of reductase is given by Equation 4-6.

$$\left.\frac{dE_{NO}}{dt}\right|_{decay} = \beta_{NO}\,E_{NO} \tag{4-6}$$

Here β_{NO} is the reductase specific decay rate. Analogously the rate of decay of oxygenase is given by

$$\left.\frac{dE_O}{dt}\right|_{decay} = \beta_O\,E_O \tag{4-7}$$

where β_O is the oxygenase specific decay rate.

For non-limiting nitrate and substrate concentration and zero dissolved oxygen concentration, the maximum value of e_{NO} results in

$$\frac{de_{NO}}{dt} = 0 = \frac{1}{X_b}\frac{dE_{NO}}{dt} - \frac{E_{NO}}{X_b^2}\frac{dX_b}{dt} \tag{4-8}$$

which yields Equation 4-9

$$e_{NO,max} = \frac{\alpha_{NO1} + \alpha_{NO2}}{\hat{\mu}_{NO} + \beta_{NO} - b} \tag{4-9}$$

Analogously the maximum value of e_O ($e_{O,max}$) under optimal conditions (non limiting dissolved oxygen and substrate concentration) is Equation 4-10

$$e_{O,ss} = \frac{\alpha_{O1} + \alpha_{O2}}{\hat{\mu}_O + \beta_O - b} \tag{4-10}$$

A summary of the model is presented in Table 4-1

Table 4-1 Summary of the model

		i					
J	Process	1. S_S	2. S_{NO}	3 X_b	4, E_O	5, E_{NO}	Process rate ρ_j, $ML^{-3}T^{-1}$
1	Aerobic growth	$-\frac{1}{Y_{C,O}}$		1			$\hat{\mu}_O\left(\frac{e_O}{e_{O,max}}\right)^{n_O}\left(\frac{S_O}{K_{O,H}+S_O}\right)\left(\frac{S_S}{K_{S,O}+S_S}\right)X_b$
2	Anoxic growth	$-\frac{1}{Y_{C,NO}}$	$\frac{1-Y_{C,NO}}{2.86Y_{C,NO}}$	1			$\mu_{NO}\left(\frac{e_{NO}}{e_{NO,max}}\right)^{n_{NO}}\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)\left(\frac{S_S}{K_{S,NO}+S_S}\right)\left(\frac{K_I}{K_I+S_O}\right)X_b$
3	Decay			-1			bX_b
4	Rate of synthesis of oxygenase				1		$\left(\alpha_{O0}+\alpha_{O1}\frac{e_O}{e_{O,max}}\right)\left(\frac{S_O}{K_{O,H}+S_O}\right)\left(\frac{S_S}{K_{S,O}+S_S}\right)X_b$
5	Rate of synthesis of reductase					1	$\left(\alpha_{NO0}+\alpha_{NO1}\frac{e_{NO}}{e_{NO,max}}\right)\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)\left(\frac{S_S}{K_{S,NO}+S_S}\right)\left(\frac{K_I}{K_I+S_O}\right)X_b$
6	Oxygenase decay rate				-1		$\beta_O E_O$
7	Reductase decay rate					-1	$\beta_{NO}E_{NO}$
Observed conversion rate $ML^{-3}T^{-1}$		$r=\sum_j \gamma_{ij}\rho_j$					

Experimental Methods

All experimental methods were performed under aseptic conditions. Freeze-dried *Pseudomonas denitrificans* (ATCC 13867) were revived in flasks containing 125 mL of nutrient broth (SIGMA.) These were shaken for two days, after which plates containing Tryptic Soy Agar (SIGMA) were streaked with revived cultures. These were grown in an incubator for three days at 35°C and then stored at 4°C in a refrigerator.

Growth experiments were conducted to observe behavior upon the transition from anoxic to aerobic, as well as aerobic to anoxic growth conditions. Experiments consisted of three phases. A nutrient solution (Table 4-2) containing limited carbon substrate (0.131 g/L of glutamic acid) was used for the initial anoxic phase. The solution contained enough carbon for bacteria to grow to an approximate absorbance of 0.1, determined by calculation and confirmed by preliminary experiments. This phase was carried out in a reactor (model BioFlo 2000, New Brunswick Scientific) to which excess nitrate (400 mg/L) was added. Bacteria were added to the reactor directly from the agar plates and grown anoxically at 30°C. Nitrogen was bubbled through the reactor to strip dissolved oxygen.

After the carbon substrate was exhausted in this anoxic phase, 500 mL of the biomass suspension was transferred to an aerated bioreactor (model F-1000, New Brunswick Scientific). The reactor contained nutrient solution with excess carbon (5 g/L glutamic acid) to allow for aerobic growth. The rate of aeration was sufficient so that dissolved oxygen concentration approached air saturation in the reactor. The length of this aerobic phase was varied.

The final anoxic phase began after the end of the aerobic phase. The contents of the reactor were diluted by adding nutrient solution containing excess carbon substrate (5 g/L glutamic acid) and nitrate (400 mg/L as N). Nitrogen gas was bubbled through the reactor to strip dissolved oxygen.

An automatic sampling system was utilized. Individual pumps withdrew culture media from the reactor and passed it through a flow cell inside a spectrophotometer (Thermo-Spectronic Genesys 10UV). The spectrophotometer was programmed to take absorbance measurements at 550 nm every 15 min. throughout all three phases.

Table 4-2. Composition of nutrient solution

	Chemicals	g/L in deionized water
Inorganic salts	NaCl	1
	NH_4Cl	1
	$MgSO_4 \cdot 7H_2O$	0.2
	$CaCl_2 \cdot 2H_2O$	0.0264
	Trace metals	1 drop[a]
Phosphates	K_2HPO_4	5
	KH_2PO_4	1.5
Carbon source	L glutamic acid	
	-limited	0.131
	-excess	5

[a] Trace metal solution containing 0.5%(w/v) each of $CuSO_4$, $FeCl_3$, $MnCl_2$ and $Na_2MoO_4 \cdot 2H_2O$.

Results and Discussion

The new simpler model was first applied to the results of experiments performed by Liu et al. (1998b), who studied the effect of length of aerobic phase on the ensuing anoxic diauxic lag of *Pseudomonas denitrificans*. As shown in Figure 4-1, a very good fit was obtained. To generate these fits, the same parameters were used for both runs (Table 4-3). For the model parameters in common with the Liu et al. (1998b) model, their values were adopted, with the exception of the initial enzyme values

Fits to two experimental runs carried out in the present research are shown in Figure 4-2 and 4-3. The runs differ in the length of the intermediate aerobic phase and concomitantly the lengths of the diauxic lags. Parameters and unmeasured initial conditions used for the fits are given in Table 4-4. A sample Excel spreadsheet used for the fits is included in Appendix A. Figures 4-2 and 4-3 show that the new model is successful in portraying carbon-limited anoxic growth, aerobic growth at nonlimiting carbon concentration and anoxic growth at nonlimiting carbon concentration. The model successfully portrays the absence of lag when switching from anoxic to aerobic conditions. It also successfully portrays the length of the diauxic lag between the aerobic phase and the final anoxic phase. Furthermore, the model is successful in showing the effect of aerobic phase length on the subsequent diauxic lag length. The experimental runs shown in Figures 4-2 and 4-3 took place on different days. Hence, the difference in initial enzyme concentrations reflected in the table would be expected. All of the parameters were identical with the exception of α_{NO2}. Forcing a common value on that parameter degraded the fits somewhat.

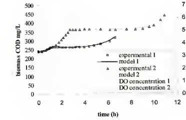


Figure 4-1 New model fit obtained for Liu et al (1998b) experimental data

Table 4-3. Parameters and unmeasured initial conditions for fit of Liu et al. (1998b) data

Parameters	Values	Units
$\hat{\mu}_O$	0.342	h^{-1}
μ_{NO}	0.322	h^{-1}
$K_{O,H}$	0.365	$g\ O_2\ m^{-3}$
K_{NO}	0.324	$g\ NO_3\text{-}N\ m^{-3}$
$K_{S,O}$	1	g COD glutamic acid m^{-3}
$K_{S,NO}$	100	g COD glutamic acid m^{-3}
K_I	0.5	$g\ O_2\ m^{-3}$
$Y_{c,O}$	0.703	g cell COD/g COD glutamic acid
$Y_{c,NO}$	0.564	g cell COD/g COD glutamic acid
α_{O1}	8.90×10^{-04}	h^{-1}
α_{O2}	1.60×10^{-06}	h^{-1}
α_{NO1}	2.27×10^{-07}	h^{-1}
α_{NO2}	3.66×10^{-05}	h^{-1}
n_O	1.6	-
n_{NO}	3.0	-
b	0	h^{-1}
β_O	0	h^{-1}
β_{NO}	0.537	h^{-1}
Unmeasured initial conditions	Values	Units
Initial e_O	1.68×10^{-04}	g enzyme COD/g cell COD
Initial e_{NO}	3.30×10^{-03}	g enzyme COD/g cell COD

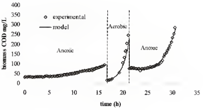


Figure 4-2 New model fit obtained for 3 phase experimental data (4.25h aerobic phase)

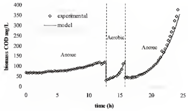


Figure 4-3 New model fit obtained for 3 phase experimental data (2.75h aerobic phase)

Table 4-4 Parameters and unmeasured initial conditions for three-phase experiments

Parameters	Figure 2 values	Figure 3 values	Units
$\hat{\mu}_O$	0.73	0.73	h^{-1}
$\hat{\mu}_{NO}$	0.336	0.336	h^{-1}
$K_{O,H}$	0.065	0.065	g O_2 m^{-3}
K_{NO}	0.77	0.77	g NO_3 N m^{-3}
$K_{S,O}$	1	1	g COD glutamic acid m^{-3}
$K_{S,NO}$	303.9	303.9	g COD glutamic acid m^{-3}
K_I	0.5	0.5	g O_2 m^{-3}
$Y_{C,O}$	0.9	0.9	g cell COD/g COD glutamic acid
$Y_{C,NO}$	0.9	0.9	g cell COD/g COD glutamic acid
α_{O1}	$3.00 \quad 10^{-03}$	$3.00 \quad 10^{-03}$	h^{-1}
α_{O2}	$3.93 \quad 10^{-03}$	$3.93 \quad 10^{-03}$	h^{-1}
α_{NO1}	$1.84 \quad 10^{-03}$	$1.84 \quad 10^{-01}$	h^{-1}
α_{NO2}	$5.38 \quad 10^{-01}$	$5.38 \quad 10^{-02}$	h^{-1}
n_O	0.5	0.5	-
n_{NO}	3	3	-
b	0.002	0.002	h^{-1}
β_O	0	0	h^{-1}
β_{NO}	0.0002	0.0002	h^{-1}
Unmeasured initial conditions	Figure 2 values	Figure 3 values	Units
Initial e_O	$9.515 \quad 10^{-3}$	0	g enzyme COD/g cell COD
Initial e_{NO}	1.375	0.2367	g enzyme COD/g cell COD

CHAPTER 5

EFFECT OF ENZYMES AND CARBON SUBSTRATE ON THE DIAUXIC LAG

It has been suggested that the periplasmic nitrate reductase present in certain bacteria plays a role in their transition from conditions where oxygen is present to conditions where oxygen is absent. To that effect, the diauxic lags of denitrifying bacteria containing the periplasmic nitrate reductase (*Paracoccus pantotrophus* formerly *Thiosphaera pantotropha*) were compared to those of denitrifying bacteria lacking the periplasmic nitrate reductase (*Pseudomonas denitrificans*) under the same experimental conditions. Furthermore, since one of the primary functions of the periplasmic nitrate reductase is redox balancing, a secondary objective was to investigate the influence of the oxidative state of the carbon substrate on the transition period of Nap-containing bacteria.

Experimental Methods

All experiments were carried out under aseptic conditions. Freeze-dried *Paracoccus pantotrophus* (ATCC 35512) and *Pseudomonas denitrificans* (ATCC 13867) were separately revived in flasks containing 125 mL of nutrient broth (SIGMA). These were placed in an incubator shaker at 35°C for one day (*Pseudomonas denitrificans* flasks) or two days (*Paracoccus pantotrophus* flasks) after which plates containing Tryptic Soy Agar (SIGMA) were streaked with revived cultures. These were placed in an incubator for three days at 35°C and then stored at 4°C in a refrigerator for no more than two weeks.

Growth experiments

A preculture phase was carried out before each experiment. A culture of *Paracoccus pantotrophus* or *Pseudomonas denitrificans* was grown aerobically at 35°C in nutrient solutions recommended for each particular culture: ATCC for *Paracoccus pantotrophus* (Table 5-1) and Kornaros et al. (1996) for *Pseudomonas denitrificans* (Table 5-2). Experiments consisted of three phases: a startup aerobic phase, a continuous aerobic phase, and a batch anoxic phase. All three phases were carried out in a fermentor (BioFlo 2000, New Brunswick Scientific).

Nutrient solution containing one of three different carbon substrates (malate, acetate, or caproate) and no nitrate was used for the startup. A specific amount of biomass from the preculture was added. The reactor was aerated and maintained at 35°C. After a short period, a feed solution containing the appropriate carbon substrate at a concentration of 1000 mg/L as COD (all other nutrients in concentrations shown in Tables 5-1 and 5-2) was pumped into the reactor to yield a dilution rate of 0.03h^{-1}. This dilution rate was used in all growth experiments, ensuring that the net specific growth rate was the same at the end of each continuous flow period. In each experiment, the culture was grown to a steady state as indicated by an absorbance (λ=550 nm, 1 cm path length) variation within 0.02%. After steady state was achieved, the feed was stopped, a portion (typically 700-800 mL) of the culture was rapidly removed, and nutrient solution containing nitrate (at the growth temperature of 35°C) was added to the reactor, giving an in situ nitrate nitrogen concentration of at least 200 mg/L. Air was substituted by nitrogen gas to strip out dissolved oxygen. Growth was monitored during this final phase by taking absorbance measurements at 550 nm every 15 minutes. Lags and specific growth rates were determined as described by Lisbon et al. (2002).

Enzyme assays

Activities of Nap and Nar were determined from a biomass sample taken near the end of the continuous aerobic phase. Cells were harvested by centrifugation (11,300 RCF for 10 minutes at 4°C) and rinsed with 20 mM Tris buffer (pH 7). They were resuspended in 2 mL of the buffer. The assay method used was a modification of that given by Jones et al. (1977) that measures the activity of nitrate reductase in intact cells based on the rate at which reduced benzyl viologen (BV) decolorizes, which is proportional to the rate of electron transfer to nitrate. Inside an anaerobic chamber (Coy Laboratories Type 'A'), several 3mm glass beads were added (for mixing) to a 1 cm optical path borosilicate cuvette. The cuvette was filled halfway with a solution of benzyl viologen and Tris buffer (0.3 mM BV, 20 mM Tris HCl). 200 µL of resuspended biomass were added (giving a final bacteria concentration of approximately 55 mg/L dry weight). Enough dithionite (usually 25 µL of a 25 mM solution) was added to reduce the benzyl viologen to give an absorbance of approximately 1.8 at 550 nm. More benzyl viologen/Tris solution was added to completely fill the headspace of the cuvette. The cuvette was then stoppered with a Wheaton seal and placed in a spectrophotometer (Thermo-Spectronic Genesys 10UV) programmed to take automatic measurements every 15 seconds. After monitoring the absorbance for 3 minutes to give a baseline, the reaction was initiated by injecting nitrate through the rubber seal to give a final concentration of 6 mM. The cuvette was inverted twice for mixing and returned to the spectrophotometer, where the absorbance was monitored for 7 minutes. The seal was then broken, air was allowed into the cuvette to complete the oxidation (decolorization) of benzyl viologen, and the remaining absorbance (absorbance of biomass alone) was measured. The slope of the absorbance vs time plot was converted to units of benzyl viologen oxidized per time, which would be equivalent to the enzyme's ability to catalyze the use reduction of nitrate. The unit conversion was done as shown in Equation 5-1. Assuming we are within the linear range, we use

$$\frac{m * C_v}{max - base} \tag{5-1}$$

where *m* is the slope of decolorization, *Cv* is the benzyl viologen concentration, max is the maximum absorbance (which includes biomass and reduced benzyl viologen absorbance), and *base* is the absorbance of biomass only. In order to obtain the specific activity (concentration change per biomass, Equation 5-1 was then divided by biomass concentration (in units of mg/L biomass).

In order to distinguish between activity of Nap and activity of Nar, two assays were performed simultaneously, one containing 100μM azide and one without azide.

Table 5-1: Composition of nutrient solution for *Paracoccus pantotrophus*

	Chemicals	g/L in deionized water
Inorganic salts	NH_4Cl	0.3
	$MgSO_4 \cdot 7H_2O$	0.1
	Vishniac and Santer Trace Element Solution	2 mL
Phosphates	Na_2HPO_4	4.2
	KH_2PO_4	1.5
Carbon source	-D-L malic acid	1.297
	Caproic acid	0.454 mL
	Sodium acetate	1.36

Table 5.2 Composition of nutrient solution for *Pseudomonas denitrificans*

	Chemicals	g/L in deionized water
Inorganic salts	NaCl	1
	NH_4Cl	1
	$MgSO_4 \cdot 7H_2O$	0.2
	$CaCl_2 \cdot 2H_2O$	0.0264
	Trace metals	1 drop[a]
Phosphates	K_2HPO_4	5
	KH_2PO_4	1.5
Carbon source	DL-malic acid	6.45
	Sodium acetate	6.77

[a] Trace metal solutions containing 0.5%(w/v) each of $CuSO_4$, $FeCl_3$, $MnCl_2$ and $Na_2MoO_4 \cdot 2H_2O$

Data Analysis

In 1987, the International Association on Water Quality (IAWQ) (now International Water Association) published the activated sludge model (ASM) No. 1. In this model, the process rate expression given for aerobic growth of heterotrophs is given by

$$\frac{dX_{B,H}}{dt} = \hat{\mu}_H \left(\frac{S_S}{K_S + S_S} \right) \left(\frac{S_O}{K_{O,H} + S_O} \right) X_{B,H} \tag{5-2}$$

where $\hat{\mu}_H$ = maximum specific growth rate coefficient for heterotrophs, S_S = soluble substrate concentration, K_S = half-saturation coefficient for substrate, S_O = dissolved oxygen concentration, $K_{O,H}$ = half-saturation coefficient for oxygen for heterotrophs, and $X_{B,H}$ = active heterotrophic biomass concentration. If excess carbon and dissolved oxygen concentrations are used, this equation can be approximated by Equation 5-3

$$\frac{dX_{B,H}}{dt} = \hat{\mu}_H X_{B,H} \tag{5-3}$$

Integrating this equation, the following is obtained

$$X_{B,H}(t) = X_{B,H}(0)\, e^{\hat{\mu}_H t} \tag{5-4}$$

Because biomass absorbance is directly proportional to biomass concentration, an analogous expression applies for biomass absorbance

$$A = A_O e^{\mu_{max}(t - t_O)} \tag{5-5}$$

or

$$\ln A = \left(\ln A_O - \mu_{max} t_O\right) + \mu_{max} t \tag{5-6}$$

Here A = biomass absorbance, t_O = time at the beginning of growth in the aerobic phase, A_O = absorbance at the beginning of the aerobic phase, μ_{max} = maximum aerobic specific growth rate (formerly $\hat{\mu}_H$)

Under anoxic conditions, if nitrate is in excess, as is the case at the beginning of the anoxic phase, an analogous equation to 5-6 applies:

$$\ln A = \left(\ln A_L - \mu_{max,N}\, t_L\right) + \mu_{max,N}\, t \tag{5-7}$$

where A = biomass absorbance, t_L = time at end of diauxic lag, A_L = average absorbance during the lag, $\mu_{max,N}$ = maximum anoxic specific growth rate.

In order to calculate the maximum specific growth rate in the anoxic phase, ln (absorbance) vs. time was plotted. Some logarithmic plots are included in Appendix B. A straight line is fitted and the slope, according to Equation 5-7, yields the maximum specific growth rate for anoxic conditions. The end of the lag phase, t_L, is determined from the intersection of a line of zero slope drawn at the average absorbance during the lag, A_L, and the straight line for exponential growth at the beginning of the anoxic phase. The diauxic lag, t_D, is obtained from

$$t_D = t_L - t_S \tag{5-8}$$

where t_S is the time at which air was turned off

In some experiments the semi-logarithmic plot showed that during the second anoxic phase there were two phases of exponential growth, each with different growth rate. In these cases, two straight lines were fit and the intersection of the two lines was the end of the "lag."

Results and Discussion

Figure 5-1 presents the results of *Pseudomonas denitrificans* growing on acetate. The beginning of the graph shows the transition from the startup phase to the continuous phase. The absorbance then becomes approximately constant as the culture approaches steady state. It can be seen that, after switching from the aerobic continuous culture to anoxic batch conditions, the culture experienced a diauxic lag of 16 hours before resuming exponential growth. Figure 5-2 shows the results with *Paracoccus pantotrophus* growing with the same substrate. As before, the culture was switched from aerobic continuous flow to anoxic batch conditions after reaching steady state. An enlargement of the anoxic batch phase is shown within Figure 5-2. From this view it can be seen that, after switching from the aerobic continuous culture to anoxic batch conditions, for a period of approximately 1 hour the specific growth rate was relatively slow (0.13 h^{-1}) after which it increased to a higher value (0.21 h^{-1}). The change in growth rate is easier to observe in the semi-logarithmic plot in Appendix B (Figure B-2). It is debatable whether or not this should be considered a diauxic lag. Traditionally a diauxic lag has been defined as a period of little or no growth (Monod, 1942). In the present case the initial growth rate is 62% of the ultimate growth rate. Whether or not this is to be called a diauxic lag, the period over which the rate was slower is short.

Experiments were repeated utilizing a more oxidized carbon substrate (malate). Results from these experiments are shown in Figures 5-3 and 5-4. Once again, results with *Pseudomonas denitrificans* showed a long diauxic lag (5 h) whereas a short lag (0.5 h) was observed with *Paracoccus pantotrophus* (Figures B-3 and B-4 in Appendix B).

In order to verify the effect of carbon substrate, an experiment was done with *Paracoccus pantotrophus* using caproate as carbon substrate. Results are shown in Figure 5-5 and the semi logarithmic plot is included in Figure B-5 in Appendix B. After switching from aerobic to anoxic conditions, the biomass concentration increased immediately at a constant specific growth rate (no lag). *Pseudomonas denitrificans* was unable to grow using caproate as carbon substrate.

It was expected for *Paracoccus pantotrophus* that caproate would result in a shorter lag, followed by acetate and then malate. Clearly, caproate had no lag and clearly malate had some lag, albeit a short one. As mentioned above, it is debatable as to whether or not acetate displayed a lag. If it did, it was somewhat longer than the one for malate but less severe. Table 5-3 shows a summary of the anoxic specific growth rates and diauxic lag lengths for each experiment as calculated from the semi-logarithmic plots in Appendix B.

Enzyme assays were performed at the end of the continuous aerobic phase using reduced benzyl viologen as electron donor. Because benzyl viologen is highly permeable to the cell membrane in intact cells (Bedzyk et al., 1999), the activities measured in these assays include the effect of Nar and Nap combined. Because at micromolar concentrations azide inhibits Nar and has no effect on Nap, this inhibitor can be used to distinguish between the two enzymes. In all experiments activities measured with and without azide gave close to identical values meaning that the effect of Nap was the only one being measured. Because oxygen is known to inhibit Nar and these samples were taken after a long period of aerobic growth, no Nar activity was expected. On experiments performed with *Paracoccus pantotrophus*, the activities measured in the assays were quite high compared to those obtained with *Pseudomonas denitrificans*, which were practically zero (Table 5-4). This is consistent with the observed longer lags with *Pseudomonas denitrificans*. Enzyme activities showed little variation with the nature of the carbon substrate used for growth.

As expected, cultures able to express Nap had much shorter (if any) lags than those that only express Nar upon switching from aerobic to anoxic conditions, thus suggesting that Nap has an important role in this transition.

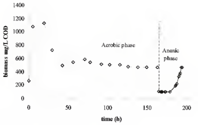


Figure 5-1 *Pseudomonas denitrificans* in acetate

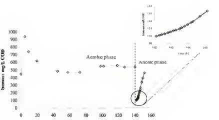


Figure 5-2 *Paracoccus pantotrophus* in acetate

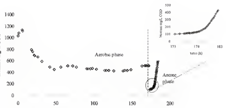


Figure 5-3 *Pseudomonas denitrificans* in malate

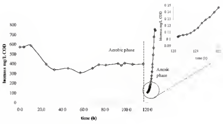


Figure 5-4 *Paracoccus pantotrophus* in malate.

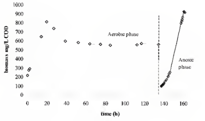


Figure 5-5 Paracoccus pantotrophus in caproate.

Table 5-3 Summary of experimental results

Experiment	Initial growth rate (h^{-1})	Final growth rate (h^{-1})	Diauxic lag length (h)
Paracoccus pantotrophus in caproate	0.11	0.11	0
Paracoccus pantotrophus in malate	0.07	0.33	0.5
Paracoccus pantotrophus in acetate	0.13	0.21	0 or 1
Pseudomonas denitrificans in malate	0.05	0.28	5
Pseudomonas denitrificans in acetate	0.00	0.13	16

Table 5-4 Summary of enzyme assay results

Experiment	Activity without azide	Activity with azide
Paracoccus pantotrophus in caproate	1.61E-11	1.54E-11
Paracoccus pantotrophus in malate	1.77E-11	1.74E-11
Paracoccus pantotrophus in acetate	8.38E-12	8.25E-12
Pseudomonas denitrificans in malate	1.35E-13	-
Pseudomonas denitrificans in acetate	8.69E-14	1.69E-13

A Preliminary Model

The simple model presented in Chapter 4 was modified to incorporate the effect of the periplasmic nitrate reductase The new anoxic growth rate expression is given by Equation 5-9

$$\left.\frac{dX_b}{dt}\right|_{anoxic} = \left[P+\mu_n\left(\frac{e_n}{e_{n,max}}\right)^{n_n}\right]\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)\left(\frac{S_S}{K_{S,NO}+S_S}\right)\left(\frac{K_I}{K_I+S_O}\right)X_b \quad (5\text{-}9)$$

where μ_n is the maximum specific growth rate due to Nar, e_n is Nar specific level and $e_{n,max}$ is Nar maximum specific level. A constant tunable term, P, was added to account for presence of Nap For bacteria lacking Nap, P is zero and the expression for anoxic growth rate reduces to its original form (Equation 4-1) All other expressions in the model were kept as described in Chapter 4 The enzyme synthesis and enzyme decay expressions continue to represent only Nar

Experimental data was fit using this model. Figures 5-6 and 5-7 show fits to the experiments performed using acetate as electron donor The model was able to capture the lag observed with *Pseudomonas denitrificans* and the "lack of lag" observed with *Paracoccus pantotrophus* Different parameters were used for both experiments, since different bacterial species were used Table 5-5 shows the parameters and initial conditions used The values used for μ_n were those obtained experimentally Fits to the rest of the experiments are shown in Appendix C

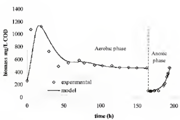


Figure 5.6 Fit obtained with *Pseudomonas denitrificans* in acetate.

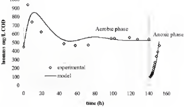


Figure 5-7 Fit obtained with *Paracoccus pantotrophus* in acetate

Table 5-5 Parameters and unmeasured initial conditions

Parameters	Figure 9 values	Figure 10 values	Units
μ_O	0.298	0.24	h^{-1}
$\hat{\mu}_m$	0.128	0.209	h^{-1}
$K_{O,H}$	0.129	0.129	g O_2 m^{-3}
K_{NO}	0.129	0.129	g NO_3-N m^{-3}
$K_{s,O}$	421.9	242.9	g COD glutamic acid m^{-3}
$K_{s,NO}$	100	100	g COD glutamic acid m^{-3}
K_I	0.5	0.5	g O_2 m^{-3}
$Y_{c,O}$	0.9	0.9	g cell COD/g COD glutamic acid
$Y_{c,NO}$	1	1	g cell COD/g COD glutamic acid
P	0	$1.34 \quad 10^{-04}$	h^{-1}
α_{O1}	0	0	h^{-1}
α_{O2}	$5.35 \quad 10^{-03}$	$5.88 \quad 10^{-03}$	h^{-1}
α_{NO1}	$8.00 \quad 10^{-06}$	$8.00 \quad 10^{-05}$	h^{-1}
α_{NO2}	$3.48 \quad 10^{-04}$	$3.48 \quad 10^{-04}$	h^{-1}
n_O	2.57	1.82	-
n_{NO}	1	1.80	
b	0	0	h^{-1}
β_O	0.274	0.318	h^{-1}
β_{NO}	0.5	0.5	h^{-1}
Unmeasured initial conditions	Figure 9 values	Figure 10 values	Units
Initial e_O	$9.35 \quad 10^{-3}$	0.0106	g enzyme COD/g biomass COD
Initial e_{NO}	$3.08 \quad 10^{-15}$	$1.81 \quad 10^{-14}$	g enzyme COD/g biomass COD

CHAPTER 6
CONCLUSIONS

A simplified model of the diauxic growth of bacteria switching between electron acceptors has been developed. Based on the application of this model to new experimental data generated as part of this study, as well as data from the literature, the following conclusions may be drawn:

- The model provides excellent fits to diauxic growth of heterotrophic bacteria switching between electron acceptors.
- The model is capable of portraying denitrification dynamics in cyclic anoxic/aerobic environments.
- The model is capable of simulating carbon limited growth.

Based on the data obtained from growth experiments using denitrifying bacteria containing a periplasmic nitrate reductase (*Paracoccus pantotrophus*) and denitrifying bacteria containing only a membrane-bound nitrate reductase (*Pseudomonas denitrificans*), the following conclusions may be drawn:

- The periplasmic nitrate reductase may have a role in speeding the transition from growth with oxygen as terminal electron acceptor to growth with nitrate as terminal electron acceptor.
- There appears to be a correlation between the oxidation state of the carbon substrate and the length of the diauxic lag.

CHAPTER 7
FUTURE WORK

Many aspects of diauxic behavior when switching between electron acceptors are still a mystery. Considerable research must still be done for better understanding of this phenomenon.

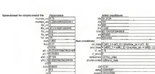
One key question yet to be answered is how the carbon substrate concentration affects the length of the diauxic lag. Experiments described in Chapter 5 where cultures were grown continuously under aerobic conditions and then switched to batch anoxic conditions provided low substrate concentrations during the continuous phase and resulted in short or non-existent diauxic lags for Nap-containing bacteria. On the other hand, experiments not shown in this dissertation where Nap-containing bacteria were grown under aerobic, batch, excess substrate conditions and then switched to batch anoxic conditions resulted in slightly longer lags (as long as 3 hours). Ellington et al. (2003) studied Nap expression in continuous cultures of *Paracoccus pantotrophus* in different carbon substrates in limited concentrations and observed that levels of expression were higher than those in batch cultures. Thus it seems that carbon substrate concentration may have an effect on Nap activity, consequently affecting the length of the lag. One way to examine the effect of carbon substrate concentration is to perform continuous experiments with different dilution rates.

Our observation that Nap allows for bacteria to start growing anoxically immediately after aerobic growth leads to another unanswered question. Microbiologists state that because nitrogen reduction using Nap is not directly coupled to ATP production, it does not provide a means for growth and is only used for redox balancing. Therefore, the mechanism for growth utilizing the periplasmic enzyme must be examined. The fact that there was actual growth due to Nap was confirmed by batch experiments where Nap-containing cultures were grown anoxically with azide. Because azide inhibits Nar, the growth observed during these experiments was attributed to Nap. Bedzyk and colleagues (1999) also observed anoxic growth due to Nap. In fact, denitrification by *Pseudomonas* sp. strain G-179 was not possible without Nap. They could not define the mechanism by which energy is gained in this process. A possible explanation is that Nap allows for bacteria to quickly reduce nitrate to nitrite, and the growth observed is actually growth on nitrite instead of direct growth on nitrate. However, nitrite reductase-deficient mutants of *Pseudomonas* sp. strain G-179 can grow on nitrate (Bedzyk et al., 1999). Regardless of these findings, the hypothesis should be tested with *Paracoccus pantotrophus* by performing growth experiments using nitrite as electron acceptor instead of nitrate. If long lags are observed after switching from using oxygen to using nitrite as electron acceptor, this mechanism is not feasible.

In order to verify that, in fact, the reason why *Paracoccus pantotrophus* has no lag is because it contains Nap, it is important to perform experiments using other Nap-containing bacteria. Additionally, it would be ideal to try to inhibit Nap somehow during growth experiments using *Paracoccus pantotrophus* or to cause a mutation in this species to make it deficient in Nap and observe if this leads to longer lags.

Because the application of this study is in wastewater treatment with activated sludge, another suggestion for future work is to try to identify what percentage of bacteria in activated sludge contains Nap. Our research group is currently trying to isolate bacteria from activated sludge for this purpose. If successful in isolating cultures, diauxic growth experiments should be performed with these cultures

APPENDIX A
SAMPLE EXCEL SPREADSHEETS



Spreadsheet for simple model fits

Parameters	
mumax_nx	0.73
mumax_an	0.336049653202861
Ks_nx	1
Ks_an	20.9193091296300
Yc_nx	0.2
Yc_an	0.8
Yn_an	15
Kab	0.868
Kno	0.77
b	0.092
aN_1	0.772592038478531
aN_2	0.28393
aNO_1	0.154
aNO_2	0.533738254264156
bO	5
bM3	0.0002
z	0.5
Se	5

Initial conditions	
X0	38.3728
Sa	141
Sn	400
Ea	0.228
Enn	43.754228794086
ao	=Ea/X0
ano	=Enn/X0

Run conditions	
ao_max	=(aO_1 + aO_2) / (mumax_nx + aO_b)
ano_max	=(aNO_1 + aNO_2) / (mumax_an + bNO_b)
t_rat	0.5
n_steps	2
totconsump	=D7/ao_max
anotconsump	=D8/ano_max
D	=0.01
delta	=D35/D87
deltat	=D100/D90

sum errors squared =SUM(G27:G157)

t	t elapsed	abs
11.5	=A22-A22	0.031
11.75	=A23-A22	0.032
12	=A24-A22	0.031
12.25	=A25-A22	0.032
12.5	=A26-A22	0.032
12.75	=A27-A22	0.032
13	=A28-A22	0.031
13.25	=A29-A22	0.031
13.5	=A30-A22	0.031
13.75	=A31-A22	0.032
14	=A32-A22	0.033
14.25	=A33-A22	0.032
14.5	=A34-A22	0.034
14.75	=A35-A22	0.033
15	=A36-A22	0.032
15.25	=A37-A22	0.031
15.5	=A38-A22	0.032
15.75	=A39-A22	0.035
16	=A40-A22	0.037
16.25	=A41-A22	0.036
16.5	=A42-A22	0.034
16.75	=A43-A22	0.035
17	=A44-A22	0.034
17.25	=A45-A22	0.035
17.5	=A46-A22	0.035
17.75	=A47-A22	0.035
18	=A48-A22	0.036
18.25	=A49-A22	0.036
18.5	=A50-A22	0.032
18.75	=A51-A22	0.037
19	=A52-A22	0.039
19.25	=A53-A22	0.041
19.5	=A54-A22	0.042
19.75	=A55-A22	0.043
20	=A56-A22	0.042
20.25	=A57-A22	0.044
20.5	=A58-A22	0.046
20.75	=A59-A22	0.047
21	=A60-A22	0.048
21.25	=A61-A22	0.045
21.5	=A62-A22	0.049
21.75	=A63-A22	0.051
22	=A64-A22	0.054
22.25	=A65-A22	0.054
22.5	=A66-A22	0.057
22.75	=A67-A22	0.06
23	=A68-A22	0.061
23.25	=A69-A22	0.063
23.5	=A70-A22	0.064
23.75	=A71-A22	0.064
24	=A72-A22	0.063

mg/L hm	t elapsed	Xh, measured
=C22*690*1.42	0	=D22
=C23*690*1.42	0.01	
=C24*690*1.42	0.02	
=C25*690*1.42	0.03	
=C26*690*1.42	0.04	
=C27*690*1.42	0.05	
=C28*690*1.42	0.06	
=C29*690*1.42	0.07	
=C30*690*1.42	0.08	
=C31*690*1.42	0.09	
=C32*690*1.42	0.1	
=C33*690*1.42	0.11	
=C34*690*1.42	0.12	
=C35*690*1.42	0.13	
=C36*690*1.42	0.14	
=C37*690*1.42	0.15	
=C38*690*1.42	0.16	
=C39*690*1.42	0.17	
=C40*690*1.42	0.18	
=C41*690*1.42	0.19	
=C42*690*1.42	0.2	
=C43*690*1.42	0.21	
=C44*690*1.42	0.22	
=C45*690*1.42	0.23	
=C46*690*1.42	0.24	
=C47*690*1.42	0.25	=D23
=C48*690*1.42	0.26	
=C49*690*1.42	0.27	
=C50*690*1.42	0.28	
=C51*690*1.42	0.29	
=C52*690*1.42	0.3	
=C53*690*1.42	0.31	
=C54*690*1.42	0.32	
=C55*690*1.42	0.33	
=C56*690*1.42	0.34	
=C57*690*1.42	0.35	
=C58*690*1.42	0.36	
=C59*690*1.42	0.37	
=C60*690*1.42	0.38	
=C61*690*1.42	0.39	
=C62*690*1.42	0.4	
=C63*690*1.42	0.41	
=C64*690*1.42	0.42	
=C65*690*1.42	0.43	
=C66*690*1.42	0.44	
=C67*690*1.42	0.45	
=C68*690*1.42	0.46	
=C69*690*1.42	0.47	
=C70*690*1.42	0.48	
=C71*690*1.42	0.49	
=C72*690*1.42	0.5	=D24

error^2	Xb	Ss
=IF(F22<>"",(F22-H22)^2,"")	=Xb	=Ss
=IF(F23<>"",(F23-H23)^2,"")	=H22+Dt*T22	=MAX(I22+Dt*U22,0)
=IF(F24<>"",(F24-H24)^2,"")	=H23+Dt*T23	=MAX(I23+Dt*U23,0)
=IF(F25<>"",(F25-H25)^2,"")	=H24+Dt*T24	=MAX(I24+Dt*U24,0)
=IF(F26<>"",(F26-H26)^2,"")	=H25+Dt*T25	=MAX(I25+Dt*U25,0)
=IF(F27<>"",(F27-H27)^2,"")	=H26+Dt*T26	=MAX(I26+Dt*U26,0)
=IF(F28<>"",(F28-H28)^2,"")	=H27+Dt*T27	=MAX(I27+Dt*U27,0)
=IF(F29<>"",(F29-H29)^2,"")	=H28+Dt*T28	=MAX(I28+Dt*U28,0)
=IF(F30<>"",(F30-H30)^2,"")	=H29+Dt*T29	=MAX(I29+Dt*U29,0)
=IF(F31<>"",(F31-H31)^2,"")	=H30+Dt*T30	=MAX(I30+Dt*U30,0)
=IF(F32<>"",(F32-H32)^2,"")	=H31+Dt*T31	=MAX(I31+Dt*U31,0)
=IF(F33<>"",(F33-H33)^2,"")	=H32+Dt*T32	=MAX(I32+Dt*U32,0)
=IF(F34<>"",(F34-H34)^2,"")	=H33+Dt*T33	=MAX(I33+Dt*U33,0)
=IF(F35<>"",(F35-H35)^2,"")	=H34+Dt*T34	=MAX(I34+Dt*U34,0)
=IF(F36<>"",(F36-H36)^2,"")	=H35+Dt*T35	=MAX(I35+Dt*U35,0)
=IF(F37<>"",(F37-H37)^2,"")	=H36+Dt*T36	=MAX(I36+Dt*U36,0)
=IF(F38<>"",(F38-H38)^2,"")	=H37+Dt*T37	=MAX(I37+Dt*U37,0)
=IF(F39<>"",(F39-H39)^2,"")	=H38+Dt*T38	=MAX(I38+Dt*U38,0)
=IF(F40<>"",(F40-H40)^2,"")	=H39+Dt*T39	=MAX(I39+Dt*U39,0)
=IF(F41<>"",(F41-H41)^2,"")	=H40+Dt*T40	=MAX(I40+Dt*U40,0)
=IF(F42<>"",(F42-H42)^2,"")	=H41+Dt*T41	=MAX(I41+Dt*U41,0)
=IF(F43<>"",(F43-H43)^2,"")	=H42+Dt*T42	=MAX(I42+Dt*U42,0)
=IF(F44<>"",(F44-H44)^2,"")	=H43+Dt*T43	=MAX(I43+Dt*U43,0)
=IF(F45<>"",(F45-H45)^2,"")	=H44+Dt*T44	=MAX(I44+Dt*U44,0)
=IF(F46<>"",(F46-H46)^2,"")	=H45+Dt*T45	=MAX(I45+Dt*U45,0)
=IF(F47<>"",(F47-H47)^2,"")	=H46+Dt*T46	=MAX(I46+Dt*U46,0)
=IF(F48<>"",(F48-H48)^2,"")	=H47+Dt*T47	=MAX(I47+Dt*U47,0)
=IF(F49<>"",(F49-H49)^2,"")	=H48+Dt*T48	=MAX(I48+Dt*U48,0)
=IF(F50<>"",(F50-H50)^2,"")	=H49+Dt*T49	=MAX(I49+Dt*U49,0)
=IF(F51<>"",(F51-H51)^2,"")	=H50+Dt*T50	=MAX(I50+Dt*U50,0)
=IF(F52<>"",(F52-H52)^2,"")	=H51+Dt*T51	=MAX(I51+Dt*U51,0)
=IF(F53<>"",(F53-H53)^2,"")	=H52+Dt*T52	=MAX(I52+Dt*U52,0)
=IF(F54<>"",(F54-H54)^2,"")	=H53+Dt*T53	=MAX(I53+Dt*U53,0)
=IF(F55<>"",(F55-H55)^2,"")	=H54+Dt*T54	=MAX(I54+Dt*U54,0)
=IF(F56<>"",(F56-H56)^2,"")	=H55+Dt*T55	=MAX(I55+Dt*U55,0)
=IF(F57<>"",(F57-H57)^2,"")	=H56+Dt*T56	=MAX(I56+Dt*U56,0)
=IF(F58<>"",(F58-H58)^2,"")	=H57+Dt*T57	=MAX(I57+Dt*U57,0)
=IF(F59<>"",(F59-H59)^2,"")	=H58+Dt*T58	=MAX(I58+Dt*U58,0)
=IF(F60<>"",(F60-H60)^2,"")	=H59+Dt*T59	=MAX(I59+Dt*U59,0)
=IF(F61<>"",(F61-H61)^2,"")	=H60+Dt*T60	=MAX(I60+Dt*U60,0)
=IF(F62<>"",(F62-H62)^2,"")	=H61+Dt*T61	=MAX(I61+Dt*U61,0)
=IF(F63<>"",(F63-H63)^2,"")	=H62+Dt*T62	=MAX(I62+Dt*U62,0)
=IF(F64<>"",(F64-H64)^2,"")	=H63+Dt*T63	=MAX(I63+Dt*U63,0)
=IF(F65<>"",(F65-H65)^2,"")	=H64+Dt*T64	=MAX(I64+Dt*U64,0)
=IF(F66<>"",(F66-H66)^2,"")	=H65+Dt*T65	=MAX(I65+Dt*U65,0)
=IF(F67<>"",(F67-H67)^2,"")	=H66+Dt*T66	=MAX(I66+Dt*U66,0)
=IF(F68<>"",(F68-H68)^2,"")	=H67+Dt*T67	=MAX(I67+Dt*U67,0)
=IF(F69<>"",(F69-H69)^2,"")	=H68+Dt*T68	=MAX(I68+Dt*U68,0)
=IF(F70<>"",(F70-H70)^2,"")	=H69+Dt*T69	=MAX(I69+Dt*U69,0)
=IF(F71<>"",(F71-H71)^2,"")	=H70+Dt*T70	=MAX(I70+Dt*U70,0)
=IF(F72<>"",(F72-H72)^2,"")	=H71+Dt*T71	=MAX(I71+Dt*U71,0)

Sn	Eo	Eno
=Sn	=Eo	=Eno
=MAX(J22+Dt*V22 0)	=K22+Dt*W22	=L22+Dt*X22
=MAX(J23+Dt*V23 0)	=K23+Dt*W23	=L23+Dt*X23
=MAX(J24+Dt*V24 0)	=K24+Dt*W24	=L24+Dt*X24
=MAX(J25+Dt*V25 0)	=K25+Dt*W25	=L25+Dt*X25
=MAX(J26+Dt*V26 0)	=K26+Dt*W26	=L26+Dt*X26
=MAX(J27+Dt*V27 0)	=K27+Dt*W27	=L27+Dt*X27
=MAX(J28+Dt*V28 0)	=K28+Dt*W28	=L28+Dt*X28
=MAX(J29+Dt*V29 0)	=K29+Dt*W29	=L29+Dt*X29
=MAX(J30+Dt*V30 0)	=K30+Dt*W30	=L30+Dt*X30
=MAX(J31+Dt*V31 0)	=K31+Dt*W31	=L31+Dt*X31
=MAX(J32+Dt*V32 0)	=K32+Dt*W32	=L32+Dt*X32
=MAX(J33+Dt*V33 0)	=K33+Dt*W33	=L33+Dt*X33
=MAX(J34+Dt*V34 0)	=K34+Dt*W34	=L34+Dt*X34
=MAX(J35+Dt*V35 0)	=K35+Dt*W35	=L35+Dt*X35
=MAX(J36+Dt*V36 0)	=K36+Dt*W36	=L36+Dt*X36
=MAX(J37+Dt*V37 0)	=K37+Dt*W37	=L37+Dt*X37
=MAX(J38+Dt*V38 0)	=K38+Dt*W38	=L38+Dt*X38
=MAX(J39+Dt*V39 0)	=K39+Dt*W39	=L39+Dt*X39
=MAX(J40+Dt*V40 0)	=K40+Dt*W40	=L40+Dt*X40
=MAX(J41+Dt*V41 0)	=K41+Dt*W41	=L41+Dt*X41
=MAX(J42+Dt*V42 0)	=K42+Dt*W42	=L42+Dt*X42
=MAX(J43+Dt*V43 0)	=K43+Dt*W43	=L43+Dt*X43
=MAX(J44+Dt*V44 0)	=K44+Dt*W44	=L44+Dt*X44
=MAX(J45+Dt*V45 0)	=K45+Dt*W45	=L45+Dt*X45
=MAX(J46+Dt*V46 0)	=K46+Dt*W46	=L46+Dt*X46
=MAX(J47+Dt*V47 0)	=K47+Dt*W47	=L47+Dt*X47
=MAX(J48+Dt*V48 0)	=K48+Dt*W48	=L48+Dt*X48
=MAX(J49+Dt*V49 0)	=K49+Dt*W49	=L49+Dt*X49
=MAX(J50+Dt*V50 0)	=K50+Dt*W50	=L50+Dt*X50
=MAX(J51+Dt*V51 0)	=K51+Dt*W51	=L51+Dt*X51
=MAX(J52+Dt*V52 0)	=K52+Dt*W52	=L52+Dt*X52
=MAX(J53+Dt*V53 0)	=K53+Dt*W53	=L53+Dt*X53
=MAX(J54+Dt*V54 0)	=K54+Dt*W54	=L54+Dt*X54
=MAX(J55+Dt*V55 0)	=K55+Dt*W55	=L55+Dt*X55
=MAX(J56+Dt*V56 0)	=K56+Dt*W56	=L56+Dt*X56
=MAX(J57+Dt*V57 0)	=K57+Dt*W57	=L57+Dt*X57
=MAX(J58+Dt*V58 0)	=K58+Dt*W58	=L58+Dt*X58
=MAX(J59+Dt*V59 0)	=K59+Dt*W59	=L59+Dt*X59
=MAX(J60+Dt*V60 0)	=K60+Dt*W60	=L60+Dt*X60
=MAX(J61+Dt*V61 0)	=K61+Dt*W61	=L61+Dt*X61
=MAX(J62+Dt*V62 0)	=K62+Dt*W62	=L62+Dt*X62
=MAX(J63+Dt*V63 0)	=K63+Dt*W63	=L63+Dt*X63
=MAX(J64+Dt*V64 0)	=K64+Dt*W64	=L64+Dt*X64
=MAX(J65+Dt*V65 0)	=K65+Dt*W65	=L65+Dt*X65
=MAX(J66+Dt*V66 0)	=K66+Dt*W66	=L66+Dt*X66
=MAX(J67+Dt*V67 0)	=K67+Dt*W67	=L67+Dt*X67
=MAX(J68+Dt*V68 0)	=K68+Dt*W68	=L68+Dt*X68
=MAX(J69+Dt*V69 0)	=K69+Dt*W69	=L69+Dt*X69
=MAX(J70+Dt*V70 0)	=K70+Dt*W70	=L70+Dt*X70
=MAX(J71+Dt*V71 0)	=K71+Dt*W71	=L71+Dt*X71

eo	eno	Sc
=K22/H22	=L22/H22	0
=K23/H23	=L23/H23	0
=K24/H24	=L24/H24	0
=K25/H25	=L25/H25	0
=K26/H26	=L26/H26	0
=K27/H27	=L27/H27	0
=K28/H28	=L28/H28	0
=K29/H29	=L29/H29	0
=K30/H30	=L30/H30	0
=K31/H31	=L31/H31	0
=K32/H32	=L32/H32	0
=K33/H33	=L33/H33	0
=K34/H34	=L34/H34	0
=K35/H35	=L35/H35	0
=K36/H36	=L36/H36	0
=K37/H37	=L37/H37	0
=K38/H38	=L38/H38	0
=K39/H39	=L39/H39	0
=K40/H40	=L40/H40	0
=K41/H41	=L41/H41	0
=K42/H42	=L42/H42	0
=K43/H43	=L43/H43	0
=K44/H44	=L44/H44	0
=K45/H45	=L45/H45	0
=K46/H46	=L46/H46	0
=K47/H47	=L47/H47	0
=K48/H48	=L48/H48	0
=K49/H49	=L49/H49	0
=K50/H50	=L50/H50	0
=K51/H51	=L51/H51	0
=K52/H52	=L52/H52	0
=K53/H53	=L53/H53	0
=K54/H54	=L54/H54	0
=K55/H55	=L55/H55	0
=K56/H56	=L56/H56	0
=K57/H57	=L57/H57	0
=K58/H58	=L58/H58	0
=K59/H59	=L59/H59	0
=K60/H60	=L60/H60	0
=K61/H61	=L61/H61	0
=K62/H62	=L62/H62	0
=K63/H63	=L63/H63	0
=K64/H64	=L64/H64	0
=K65/H65	=L65/H65	0
=K66/H66	=L66/H66	0
=K67/H67	=L67/H67	0
=K68/H68	=L68/H68	0
=K69/H69	=L69/H69	0
=K70/H70	=L70/H70	0
=K71/H71	=L71/H71	0
=K72/H72	=L72/H72	0

```
rate Eo
=(aO_1+aO_2*M22/eo_max)*O22/(Koh+O22)*I22/(Ks_ox+I22)*H22
=(aO_1+aO_2*M23/eo_max)*O23/(Koh+O23)*I23/(Ks_ox+I23)*H23
=(aO_1+aO_2*M24/eo_max)*O24/(Koh+O24)*I24/(Ks_ox+I24)*H24
=(aO_1+aO_2*M25/eo_max)*O25/(Koh+O25)*I25/(Ks_ox+I25)*H25
=(aO_1+aO_2*M26/eo_max)*O26/(Koh+O26)*I26/(Ks_ox+I26)*H26
=(aO_1+aO_2*M27/eo_max)*O27/(Koh+O27)*I27/(Ks_ox+I27)*H27
=(aO_1+aO_2*M28/eo_max)*O28/(Koh+O28)*I28/(Ks_ox+I28)*H28
=(aO_1+aO_2*M29/eo_max)*O29/(Koh+O29)*I29/(Ks_ox+I29)*H29
=(aO_1+aO_2*M30/eo_max)*O30/(Koh+O30)*I30/(Ks_ox+I30)*H30
=(aO_1+aO_2*M31/eo_max)*O31/(Koh+O31)*I31/(Ks_ox+I31)*H31
=(aO_1+aO_2*M32/eo_max)*O32/(Koh+O32)*I32/(Ks_ox+I32)*H32
=(aO_1+aO_2*M33/eo_max)*O33/(Koh+O33)*I33/(Ks_ox+I33)*H33
=(aO_1+aO_2*M34/eo_max)*O34/(Koh+O34)*I34/(Ks_ox+I34)*H34
=(aO_1+aO_2*M35/eo_max)*O35/(Koh+O35)*I35/(Ks_ox+I35)*H35
=(aO_1+aO_2*M36/eo_max)*O36/(Koh+O36)*I36/(Ks_ox+I36)*H36
=(aO_1+aO_2*M37/eo_max)*O37/(Koh+O37)*I37/(Ks_ox+I37)*H37
=(aO_1+aO_2*M38/eo_max)*O38/(Koh+O38)*I38/(Ks_ox+I38)*H38
=(aO_1+aO_2*M39/eo_max)*O39/(Koh+O39)*I39/(Ks_ox+I39)*H39
=(aO_1+aO_2*M40/eo_max)*O40/(Koh+O40)*I40/(Ks_ox+I40)*H40
=(aO_1+aO_2*M41/eo_max)*O41/(Koh+O41)*I41/(Ks_ox+I41)*H41
=(aO_1+aO_2*M42/eo_max)*O42/(Koh+O42)*I42/(Ks_ox+I42)*H42
=(aO_1+aO_2*M43/eo_max)*O43/(Koh+O43)*I43/(Ks_ox+I43)*H43
=(aO_1+aO_2*M44/eo_max)*O44/(Koh+O44)*I44/(Ks_ox+I44)*H44
=(aO_1+aO_2*M45/eo_max)*O45/(Koh+O45)*I45/(Ks_ox+I45)*H45
=(aO_1+aO_2*M46/eo_max)*O46/(Koh+O46)*I46/(Ks_ox+I46)*H46
=(aO_1+aO_2*M47/eo_max)*O47/(Koh+O47)*I47/(Ks_ox+I47)*H47
=(aO_1+aO_2*M48/eo_max)*O48/(Koh+O48)*I48/(Ks_ox+I48)*H48
=(aO_1+aO_2*M49/eo_max)*O49/(Koh+O49)*I49/(Ks_ox+I49)*H49
=(aO_1+aO_2*M50/eo_max)*O50/(Koh+O50)*I50/(Ks_ox+I50)*H50
=(aO_1+aO_2*M51/eo_max)*O51/(Koh+O51)*I51/(Ks_ox+I51)*H51
=(aO_1+aO_2*M52/eo_max)*O52/(Koh+O52)*I52/(Ks_ox+I52)*H52
=(aO_1+aO_2*M53/eo_max)*O53/(Koh+O53)*I53/(Ks_ox+I53)*H53
=(aO_1+aO_2*M54/eo_max)*O54/(Koh+O54)*I54/(Ks_ox+I54)*H54
=(aO_1+aO_2*M55/eo_max)*O55/(Koh+O55)*I55/(Ks_ox+I55)*H55
=(aO_1+aO_2*M56/eo_max)*O56/(Koh+O56)*I56/(Ks_ox+I56)*H56
=(aO_1+aO_2*M57/eo_max)*O57/(Koh+O57)*I57/(Ks_ox+I57)*H57
=(aO_1+aO_2*M58/eo_max)*O58/(Koh+O58)*I58/(Ks_ox+I58)*H58
=(aO_1+aO_2*M59/eo_max)*O59/(Koh+O59)*I59/(Ks_ox+I59)*H59
=(aO_1+aO_2*M60/eo_max)*O60/(Koh+O60)*I60/(Ks_ox+I60)*H60
=(aO_1+aO_2*M61/eo_max)*O61/(Koh+O61)*I61/(Ks_ox+I61)*H61
=(aO_1+aO_2*M62/eo_max)*O62/(Koh+O62)*I62/(Ks_ox+I62)*H62
=(aO_1+aO_2*M63/eo_max)*O63/(Koh+O63)*I63/(Ks_ox+I63)*H63
=(aO_1+aO_2*M64/eo_max)*O64/(Koh+O64)*I64/(Ks_ox+I64)*H64
=(aO_1+aO_2*M65/eo_max)*O65/(Koh+O65)*I65/(Ks_ox+I65)*H65
=(aO_1+aO_2*M66/eo_max)*O66/(Koh+O66)*I66/(Ks_ox+I66)*H66
=(aO_1+aO_2*M67/eo_max)*O67/(Koh+O67)*I67/(Ks_ox+I67)*H67
=(aO_1+aO_2*M68/eo_max)*O68/(Koh+O68)*I68/(Ks_ox+I68)*H68
=(aO_1+aO_2*M69/eo_max)*O69/(Koh+O69)*I69/(Ks_ox+I69)*H69
=(aO_1+aO_2*M70/eo_max)*O70/(Koh+O70)*I70/(Ks_ox+I70)*H70
=(aO_1+aO_2*M71/eo_max)*O71/(Koh+O71)*I71/(Ks_ox+I71)*H71
=(aO_1+aO_2*M72/eo_max)*O72/(Koh+O72)*I72/(Ks_ox+I72)*H72
```

rate Eno

```
=(aNO_1+aNO_2*N22/eno_max)*J22/(Kno+J22)*I22/(Ks_an+I22)*H22*Ki/(Ki+O22)
=(aNO_1+aNO_2*N23/eno_max)*J23/(Kno+J23)*I23/(Ks_an+I23)*H23*Ki/(Ki+O23)
=(aNO_1+aNO_2*N24/eno_max)*J24/(Kno+J24)*I24/(Ks_an+I24)*H24*Ki/(Ki+O24)
=(aNO_1+aNO_2*N25/eno_max)*J25/(Kno+J25)*I25/(Ks_an+I25)*H25*Ki/(Ki+O25)
=(aNO_1+aNO_2*N26/eno_max)*J26/(Kno+J26)*I26/(Ks_an+I26)*H26*Ki/(Ki+O26)
=(aNO_1+aNO_2*N27/eno_max)*J27/(Kno+J27)*I27/(Ks_an+I27)*H27*Ki/(Ki+O27)
=(aNO_1+aNO_2*N28/eno_max)*J28/(Kno+J28)*I28/(Ks_an+I28)*H28*Ki/(Ki+O28)
=(aNO_1+aNO_2*N29/eno_max)*J29/(Kno+J29)*I29/(Ks_an+I29)*H29*Ki/(Ki+O29)
=(aNO_1+aNO_2*N30/eno_max)*J30/(Kno+J30)*I30/(Ks_an+I30)*H30*Ki/(Ki+O30)
=(aNO_1+aNO_2*N31/eno_max)*J31/(Kno+J31)*I31/(Ks_an+I31)*H31*Ki/(Ki+O31)
=(aNO_1+aNO_2*N32/eno_max)*J32/(Kno+J32)*I32/(Ks_an+I32)*H32*Ki/(Ki+O32)
=(aNO_1+aNO_2*N33/eno_max)*J33/(Kno+J33)*I33/(Ks_an+I33)*H33*Ki/(Ki+O33)
=(aNO_1+aNO_2*N34/eno_max)*J34/(Kno+J34)*I34/(Ks_an+I34)*H34*Ki/(Ki+O34)
=(aNO_1+aNO_2*N35/eno_max)*J35/(Kno+J35)*I35/(Ks_an+I35)*H35*Ki/(Ki+O35)
=(aNO_1+aNO_2*N36/eno_max)*J36/(Kno+J36)*I36/(Ks_an+I36)*H36*Ki/(Ki+O36)
=(aNO_1+aNO_2*N37/eno_max)*J37/(Kno+J37)*I37/(Ks_an+I37)*H37*Ki/(Ki+O37)
=(aNO_1+aNO_2*N38/eno_max)*J38/(Kno+J38)*I38/(Ks_an+I38)*H38*Ki/(Ki+O38)
=(aNO_1+aNO_2*N39/eno_max)*J39/(Kno+J39)*I39/(Ks_an+I39)*H39*Ki/(Ki+O39)
=(aNO_1+aNO_2*N40/eno_max)*J40/(Kno+J40)*I40/(Ks_an+I40)*H40*Ki/(Ki+O40)
=(aNO_1+aNO_2*N41/eno_max)*J41/(Kno+J41)*I41/(Ks_an+I41)*H41*Ki/(Ki+O41)
=(aNO_1+aNO_2*N42/eno_max)*J42/(Kno+J42)*I42/(Ks_an+I42)*H42*Ki/(Ki+O42)
=(aNO_1+aNO_2*N43/eno_max)*J43/(Kno+J43)*I43/(Ks_an+I43)*H43*Ki/(Ki+O43)
=(aNO_1+aNO_2*N44/eno_max)*J44/(Kno+J44)*I44/(Ks_an+I44)*H44*Ki/(Ki+O44)
=(aNO_1+aNO_2*N45/eno_max)*J45/(Kno+J45)*I45/(Ks_an+I45)*H45*Ki/(Ki+O45)
=(aNO_1+aNO_2*N46/eno_max)*J46/(Kno+J46)*I46/(Ks_an+I46)*H46*Ki/(Ki+O46)
=(aNO_1+aNO_2*N47/eno_max)*J47/(Kno+J47)*I47/(Ks_an+I47)*H47*Ki/(Ki+O47)
=(aNO_1+aNO_2*N48/eno_max)*J48/(Kno+J48)*I48/(Ks_an+I48)*H48*Ki/(Ki+O48)
=(aNO_1+aNO_2*N49/eno_max)*J49/(Kno+J49)*I49/(Ks_an+I49)*H49*Ki/(Ki+O49)
=(aNO_1+aNO_2*N50/eno_max)*J50/(Kno+J50)*I50/(Ks_an+I50)*H50*Ki/(Ki+O50)
=(aNO_1+aNO_2*N51/eno_max)*J51/(Kno+J51)*I51/(Ks_an+I51)*H51*Ki/(Ki+O51)
=(aNO_1+aNO_2*N52/eno_max)*J52/(Kno+J52)*I52/(Ks_an+I52)*H52*Ki/(Ki+O52)
=(aNO_1+aNO_2*N53/eno_max)*J53/(Kno+J53)*I53/(Ks_an+I53)*H53*Ki/(Ki+O53)
=(aNO_1+aNO_2*N54/eno_max)*J54/(Kno+J54)*I54/(Ks_an+I54)*H54*Ki/(Ki+O54)
=(aNO_1+aNO_2*N55/eno_max)*J55/(Kno+J55)*I55/(Ks_an+I55)*H55*Ki/(Ki+O55)
=(aNO_1+aNO_2*N56/eno_max)*J56/(Kno+J56)*I56/(Ks_an+I56)*H56*Ki/(Ki+O56)
=(aNO_1+aNO_2*N57/eno_max)*J57/(Kno+J57)*I57/(Ks_an+I57)*H57*Ki/(Ki+O57)
=(aNO_1+aNO_2*N58/eno_max)*J58/(Kno+J58)*I58/(Ks_an+I58)*H58*Ki/(Ki+O58)
=(aNO_1+aNO_2*N59/eno_max)*J59/(Kno+J59)*I59/(Ks_an+I59)*H59*Ki/(Ki+O59)
=(aNO_1+aNO_2*N60/eno_max)*J60/(Kno+J60)*I60/(Ks_an+I60)*H60*Ki/(Ki+O60)
=(aNO_1+aNO_2*N61/eno_max)*J61/(Kno+J61)*I61/(Ks_an+I61)*H61*Ki/(Ki+O61)
=(aNO_1+aNO_2*N62/eno_max)*J62/(Kno+J62)*I62/(Ks_an+I62)*H62*Ki/(Ki+O62)
=(aNO_1+aNO_2*N63/eno_max)*J63/(Kno+J63)*I63/(Ks_an+I63)*H63*Ki/(Ki+O63)
=(aNO_1+aNO_2*N64/eno_max)*J64/(Kno+J64)*I64/(Ks_an+I64)*H64*Ki/(Ki+O64)
=(aNO_1+aNO_2*N65/eno_max)*J65/(Kno+J65)*I65/(Ks_an+I65)*H65*Ki/(Ki+O65)
=(aNO_1+aNO_2*N66/eno_max)*J66/(Kno+J66)*I66/(Ks_an+I66)*H66*Ki/(Ki+O66)
=(aNO_1+aNO_2*N67/eno_max)*J67/(Kno+J67)*I67/(Ks_an+I67)*H67*Ki/(Ki+O67)
=(aNO_1+aNO_2*N68/eno_max)*J68/(Kno+J68)*I68/(Ks_an+I68)*H68*Ki/(Ki+O68)
=(aNO_1+aNO_2*N69/eno_max)*J69/(Kno+J69)*I69/(Ks_an+I69)*H69*Ki/(Ki+O69)
=(aNO_1+aNO_2*N70/eno_max)*J70/(Kno+J70)*I70/(Ks_an+I70)*H70*Ki/(Ki+O70)
=(aNO_1+aNO_2*N71/eno_max)*J71/(Kno+J71)*I71/(Ks_an+I71)*H71*Ki/(Ki+O71)
=(aNO_1+aNO_2*N72/eno_max)*J72/(Kno+J72)*I72/(Ks_an+I72)*H72*Ki/(Ki+O72)
```

```
rate aer
=mumax_ox*(M22/eo_max)^n_ox*O22/(Koh+O22)*I22/(Ks_ox+I22)*H22
=mumax_ox*(M23/eo_max)^n_ox*O23/(Koh+O23)*I23/(Ks_ox+I23)*H23
=mumax_ox*(M24/eo_max)^n_ox*O24/(Koh+O24)*I24/(Ks_ox+I24)*H24
=mumax_ox*(M25/eo_max)^n_ox*O25/(Koh+O25)*I25/(Ks_ox+I25)*H25
=mumax_ox*(M26/eo_max)^n_ox*O26/(Koh+O26)*I26/(Ks_ox+I26)*H26
=mumax_ox*(M27/eo_max)^n_ox*O27/(Koh+O27)*I27/(Ks_ox+I27)*H27
=mumax_ox*(M28/eo_max)^n_ox*O28/(Koh+O28)*I28/(Ks_ox+I28)*H28
=mumax_ox*(M29/eo_max)^n_ox*O29/(Koh+O29)*I29/(Ks_ox+I29)*H29
=mumax_ox*(M30/eo_max)^n_ox*O30/(Koh+O30)*I30/(Ks_ox+I30)*H30
=mumax_ox*(M31/eo_max)^n_ox*O31/(Koh+O31)*I31/(Ks_ox+I31)*H31
=mumax_ox*(M32/eo_max)^n_ox*O32/(Koh+O32)*I32/(Ks_ox+I32)*H32
=mumax_ox*(M33/eo_max)^n_ox*O33/(Koh+O33)*I33/(Ks_ox+I33)*H33
=mumax_ox*(M34/eo_max)^n_ox*O34/(Koh+O34)*I34/(Ks_ox+I34)*H34
=mumax_ox*(M35/eo_max)^n_ox*O35/(Koh+O35)*I35/(Ks_ox+I35)*H35
=mumax_ox*(M36/eo_max)^n_ox*O36/(Koh+O36)*I36/(Ks_ox+I36)*H36
=mumax_ox*(M37/eo_max)^n_ox*O37/(Koh+O37)*I37/(Ks_ox+I37)*H37
=mumax_ox*(M38/eo_max)^n_ox*O38/(Koh+O38)*I38/(Ks_ox+I38)*H38
=mumax_ox*(M39/eo_max)^n_ox*O39/(Koh+O39)*I39/(Ks_ox+I39)*H39
=mumax_ox*(M40/eo_max)^n_ox*O40/(Koh+O40)*I40/(Ks_ox+I40)*H40
=mumax_ox*(M41/eo_max)^n_ox*O41/(Koh+O41)*I41/(Ks_ox+I41)*H41
=mumax_ox*(M42/eo_max)^n_ox*O42/(Koh+O42)*I42/(Ks_ox+I42)*H42
=mumax_ox*(M43/eo_max)^n_ox*O43/(Koh+O43)*I43/(Ks_ox+I43)*H43
=mumax_ox*(M44/eo_max)^n_ox*O44/(Koh+O44)*I44/(Ks_ox+I44)*H44
=mumax_ox*(M45/eo_max)^n_ox*O45/(Koh+O45)*I45/(Ks_ox+I45)*H45
=mumax_ox*(M46/eo_max)^n_ox*O46/(Koh+O46)*I46/(Ks_ox+I46)*H46
=mumax_ox*(M47/eo_max)^n_ox*O47/(Koh+O47)*I47/(Ks_ox+I47)*H47
=mumax_ox*(M48/eo_max)^n_ox*O48/(Koh+O48)*I48/(Ks_ox+I48)*H48
=mumax_ox*(M49/eo_max)^n_ox*O49/(Koh+O49)*I49/(Ks_ox+I49)*H49
=mumax_ox*(M50/eo_max)^n_ox*O50/(Koh+O50)*I50/(Ks_ox+I50)*H50
=mumax_ox*(M51/eo_max)^n_ox*O51/(Koh+O51)*I51/(Ks_ox+I51)*H51
=mumax_ox*(M52/eo_max)^n_ox*O52/(Koh+O52)*I52/(Ks_ox+I52)*H52
=mumax_ox*(M53/eo_max)^n_ox*O53/(Koh+O53)*I53/(Ks_ox+I53)*H53
=mumax_ox*(M54/eo_max)^n_ox*O54/(Koh+O54)*I54/(Ks_ox+I54)*H54
=mumax_ox*(M55/eo_max)^n_ox*O55/(Koh+O55)*I55/(Ks_ox+I55)*H55
=mumax_ox*(M56/eo_max)^n_ox*O56/(Koh+O56)*I56/(Ks_ox+I56)*H56
=mumax_ox*(M57/eo_max)^n_ox*O57/(Koh+O57)*I57/(Ks_ox+I57)*H57
=mumax_ox*(M58/eo_max)^n_ox*O58/(Koh+O58)*I58/(Ks_ox+I58)*H58
=mumax_ox*(M59/eo_max)^n_ox*O59/(Koh+O59)*I59/(Ks_ox+I59)*H59
=mumax_ox*(M60/eo_max)^n_ox*O60/(Koh+O60)*I60/(Ks_ox+I60)*H60
=mumax_ox*(M61/eo_max)^n_ox*O61/(Koh+O61)*I61/(Ks_ox+I61)*H61
=mumax_ox*(M62/eo_max)^n_ox*O62/(Koh+O62)*I62/(Ks_ox+I62)*H62
=mumax_ox*(M63/eo_max)^n_ox*O63/(Koh+O63)*I63/(Ks_ox+I63)*H63
=mumax_ox*(M64/eo_max)^n_ox*O64/(Koh+O64)*I64/(Ks_ox+I64)*H64
=mumax_ox*(M65/eo_max)^n_ox*O65/(Koh+O65)*I65/(Ks_ox+I65)*H65
=mumax_ox*(M66/eo_max)^n_ox*O66/(Koh+O66)*I66/(Ks_ox+I66)*H66
=mumax_ox*(M67/eo_max)^n_ox*O67/(Koh+O67)*I67/(Ks_ox+I67)*H67
=mumax_ox*(M68/eo_max)^n_ox*O68/(Koh+O68)*I68/(Ks_ox+I68)*H68
=mumax_ox*(M69/eo_max)^n_ox*O69/(Koh+O69)*I69/(Ks_ox+I69)*H69
=mumax_ox*(M70/eo_max)^n_ox*O70/(Koh+O70)*I70/(Ks_ox+I70)*H70
=mumax_ox*(M71/eo_max)^n_ox*O71/(Koh+O71)*I71/(Ks_ox+I71)*H71
=mumax_ox*(M72/eo_max)^n_ox*O72/(Koh+O72)*I72/(Ks_ox+I72)*H72
```

rate anox

```
=mumax_an*(N22/eno_max)^n_anox*J22/(Kno+J22)*I22/(Ks_an+I22)*H22*Ki/(Ki+O22)
=mumax_an*(N23/eno_max)^n_anox*J23/(Kno+J23)*I23/(Ks_an+I23)*H23*Ki/(Ki+O23)
=mumax_an*(N24/eno_max)^n_anox*J24/(Kno+J24)*I24/(Ks_an+I24)*H24*Ki/(Ki+O24)
=mumax_an*(N25/eno_max)^n_anox*J25/(Kno+J25)*I25/(Ks_an+I25)*H25*Ki/(Ki+O25)
=mumax_an*(N26/eno_max)^n_anox*J26/(Kno+J26)*I26/(Ks_an+I26)*H26*Ki/(Ki+O26)
=mumax_an*(N27/eno_max)^n_anox*J27/(Kno+J27)*I27/(Ks_an+I27)*H27*Ki/(Ki+O27)
=mumax_an*(N28/eno_max)^n_anox*J28/(Kno+J28)*I28/(Ks_an+I28)*H28*Ki/(Ki+O28)
=mumax_an*(N29/eno_max)^n_anox*J29/(Kno+J29)*I29/(Ks_an+I29)*H29*Ki/(Ki+O29)
=mumax_an*(N30/eno_max)^n_anox*J30/(Kno+J30)*I30/(Ks_an+I30)*H30*Ki/(Ki+O30)
=mumax_an*(N31/eno_max)^n_anox*J31/(Kno+J31)*I31/(Ks_an+I31)*H31*Ki/(Ki+O31)
=mumax_an*(N32/eno_max)^n_anox*J32/(Kno+J32)*I32/(Ks_an+I32)*H32*Ki/(Ki+O32)
=mumax_an*(N33/eno_max)^n_anox*J33/(Kno+J33)*I33/(Ks_an+I33)*H33*Ki/(Ki+O33)
=mumax_an*(N34/eno_max)^n_anox*J34/(Kno+J34)*I34/(Ks_an+I34)*H34*Ki/(Ki+O34)
=mumax_an*(N35/eno_max)^n_anox*J35/(Kno+J35)*I35/(Ks_an+I35)*H35*Ki/(Ki+O35)
=mumax_an*(N36/eno_max)^n_anox*J36/(Kno+J36)*I36/(Ks_an+I36)*H36*Ki/(Ki+O36)
=mumax_an*(N37/eno_max)^n_anox*J37/(Kno+J37)*I37/(Ks_an+I37)*H37*Ki/(Ki+O37)
=mumax_an*(N38/eno_max)^n_anox*J38/(Kno+J38)*I38/(Ks_an+I38)*H38*Ki/(Ki+O38)
=mumax_an*(N39/eno_max)^n_anox*J39/(Kno+J39)*I39/(Ks_an+I39)*H39*Ki/(Ki+O39)
=mumax_an*(N40/eno_max)^n_anox*J40/(Kno+J40)*I40/(Ks_an+I40)*H40*Ki/(Ki+O40)
=mumax_an*(N41/eno_max)^n_anox*J41/(Kno+J41)*I41/(Ks_an+I41)*H41*Ki/(Ki+O41)
=mumax_an*(N42/eno_max)^n_anox*J42/(Kno+J42)*I42/(Ks_an+I42)*H42*Ki/(Ki+O42)
=mumax_an*(N43/eno_max)^n_anox*J43/(Kno+J43)*I43/(Ks_an+I43)*H43*Ki/(Ki+O43)
=mumax_an*(N44/eno_max)^n_anox*J44/(Kno+J44)*I44/(Ks_an+I44)*H44*Ki/(Ki+O44)
=mumax_an*(N45/eno_max)^n_anox*J45/(Kno+J45)*I45/(Ks_an+I45)*H45*Ki/(Ki+O45)
=mumax_an*(N46/eno_max)^n_anox*J46/(Kno+J46)*I46/(Ks_an+I46)*H46*Ki/(Ki+O46)
=mumax_an*(N47/eno_max)^n_anox*J47/(Kno+J47)*I47/(Ks_an+I47)*H47*Ki/(Ki+O47)
=mumax_an*(N48/eno_max)^n_anox*J48/(Kno+J48)*I48/(Ks_an+I48)*H48*Ki/(Ki+O48)
=mumax_an*(N49/eno_max)^n_anox*J49/(Kno+J49)*I49/(Ks_an+I49)*H49*Ki/(Ki+O49)
=mumax_an*(N50/eno_max)^n_anox*J50/(Kno+J50)*I50/(Ks_an+I50)*H50*Ki/(Ki+O50)
=mumax_an*(N51/eno_max)^n_anox*J51/(Kno+J51)*I51/(Ks_an+I51)*H51*Ki/(Ki+O51)
=mumax_an*(N52/eno_max)^n_anox*J52/(Kno+J52)*I52/(Ks_an+I52)*H52*Ki/(Ki+O52)
=mumax_an*(N53/eno_max)^n_anox*J53/(Kno+J53)*I53/(Ks_an+I53)*H53*Ki/(Ki+O53)
=mumax_an*(N54/eno_max)^n_anox*J54/(Kno+J54)*I54/(Ks_an+I54)*H54*Ki/(Ki+O54)
=mumax_an*(N55/eno_max)^n_anox*J55/(Kno+J55)*I55/(Ks_an+I55)*H55*Ki/(Ki+O55)
=mumax_an*(N56/eno_max)^n_anox*J56/(Kno+J56)*I56/(Ks_an+I56)*H56*Ki/(Ki+O56)
=mumax_an*(N57/eno_max)^n_anox*J57/(Kno+J57)*I57/(Ks_an+I57)*H57*Ki/(Ki+O57)
=mumax_an*(N58/eno_max)^n_anox*J58/(Kno+J58)*I58/(Ks_an+I58)*H58*Ki/(Ki+O58)
=mumax_an*(N59/eno_max)^n_anox*J59/(Kno+J59)*I59/(Ks_an+I59)*H59*Ki/(Ki+O59)
=mumax_an*(N60/eno_max)^n_anox*J60/(Kno+J60)*I60/(Ks_an+I60)*H60*Ki/(Ki+O60)
=mumax_an*(N61/eno_max)^n_anox*J61/(Kno+J61)*I61/(Ks_an+I61)*H61*Ki/(Ki+O61)
=mumax_an*(N62/eno_max)^n_anox*J62/(Kno+J62)*I62/(Ks_an+I62)*H62*Ki/(Ki+O62)
=mumax_an*(N63/eno_max)^n_anox*J63/(Kno+J63)*I63/(Ks_an+I63)*H63*Ki/(Ki+O63)
=mumax_an*(N64/eno_max)^n_anox*J64/(Kno+J64)*I64/(Ks_an+I64)*H64*Ki/(Ki+O64)
=mumax_an*(N65/eno_max)^n_anox*J65/(Kno+J65)*I65/(Ks_an+I65)*H65*Ki/(Ki+O65)
=mumax_an*(N66/eno_max)^n_anox*J66/(Kno+J66)*I66/(Ks_an+I66)*H66*Ki/(Ki+O66)
=mumax_an*(N67/eno_max)^n_anox*J67/(Kno+J67)*I67/(Ks_an+I67)*H67*Ki/(Ki+O67)
=mumax_an*(N68/eno_max)^n_anox*J68/(Kno+J68)*I68/(Ks_an+I68)*H68*Ki/(Ki+O68)
=mumax_an*(N69/eno_max)^n_anox*J69/(Kno+J69)*I69/(Ks_an+I69)*H69*Ki/(Ki+O69)
=mumax_an*(N70/eno_max)^n_anox*J70/(Kno+J70)*I70/(Ks_an+I70)*H70*Ki/(Ki+O70)
=mumax_an*(N71/eno_max)^n_anox*J71/(Kno+J71)*I71/(Ks_an+I71)*H71*Ki/(Ki+O71)
=mumax_an*(N72/eno_max)^n_anox*J72/(Kno+J72)*I72/(Ks_an+I72)*H72*Ki/(Ki+O72)
```

fab	fes
=R22+S22-b*H22	=(-1/Yc_ox)*R22-(1/Yc_an)*S22
=R23+S23-b*H23	=-1/Yc_ox*R23-1/Yc_an*S23
=R24+S24-b*H24	=-1/Yc_ox*R24-1/Yc_an*S24
=R25+S25-b*H25	=-1/Yc_ox*R25-1/Yc_an*S25
=R26+S26-b*H26	=-1/Yc_ox*R26-1/Yc_an*S26
=R27+S27-b*H27	=-1/Yc_ox*R27-1/Yc_an*S27
=R28+S28-b*H28	=-1/Yc_ox*R28-1/Yc_an*S28
=R29+S29-b*H29	=-1/Yc_ox*R29-1/Yc_an*S29
=R30+S30-b*H30	=-1/Yc_ox*R30-1/Yc_an*S30
=R31+S31-b*H31	=-1/Yc_ox*R31-1/Yc_an*S31
=R32+S32-b*H32	=-1/Yc_ox*R32-1/Yc_an*S32
=R33+S33-b*H33	=-1/Yc_ox*R33-1/Yc_an*S33
=R34+S34-b*H34	=-1/Yc_ox*R34-1/Yc_an*S34
=R35+S35-b*H35	=-1/Yc_ox*R35-1/Yc_an*S35
=R36+S36-b*H36	=-1/Yc_ox*R36-1/Yc_an*S36
=R37+S37-b*H37	=-1/Yc_ox*R37-1/Yc_an*S37
=R38+S38-b*H38	=-1/Yc_ox*R38-1/Yc_an*S38
=R39+S39-b*H39	=-1/Yc_ox*R39-1/Yc_an*S39
=R40+S40-b*H40	=-1/Yc_ox*R40-1/Yc_an*S40
=R41+S41-b*H41	=-1/Yc_ox*R41-1/Yc_an*S41
=R42+S42-b*H42	=-1/Yc_ox*R42-1/Yc_an*S42
=R43+S43-b*H43	=-1/Yc_ox*R43-1/Yc_an*S43
=R44+S44-b*H44	=-1/Yc_ox*R44-1/Yc_an*S44
=R45+S45-b*H45	=-1/Yc_ox*R45-1/Yc_an*S45
=R46+S46-b*H46	=-1/Yc_ox*R46-1/Yc_an*S46
=R47+S47-b*H47	=-1/Yc_ox*R47-1/Yc_an*S47
=R48+S48-b*H48	=-1/Yc_ox*R48-1/Yc_an*S48
=R49+S49-b*H49	=-1/Yc_ox*R49-1/Yc_an*S49
=R50+S50-b*H50	=-1/Yc_ox*R50-1/Yc_an*S50
=R51+S51-b*H51	=-1/Yc_ox*R51-1/Yc_an*S51
=R52+S52-b*H52	=-1/Yc_ox*R52-1/Yc_an*S52
=R53+S53-b*H53	=-1/Yc_ox*R53-1/Yc_an*S53
=R54+S54-b*H54	=-1/Yc_ox*R54-1/Yc_an*S54
=R55+S55-b*H55	=-1/Yc_ox*R55-1/Yc_an*S55
=R56+S56-b*H56	=-1/Yc_ox*R56-1/Yc_an*S56
=R57+S57-b*H57	=-1/Yc_ox*R57-1/Yc_an*S57
=R58+S58-b*H58	=-1/Yc_ox*R58-1/Yc_an*S58
=R59+S59-b*H59	=-1/Yc_ox*R59-1/Yc_an*S59
=R60+S60-b*H60	=-1/Yc_ox*R60-1/Yc_an*S60
=R61+S61-b*H61	=-1/Yc_ox*R61-1/Yc_an*S61
=R62+S62-b*H62	=-1/Yc_ox*R62-1/Yc_an*S62
=R63+S63-b*H63	=-1/Yc_ox*R63-1/Yc_an*S63
=R64+S64-b*H64	=-1/Yc_ox*R64-1/Yc_an*S64
=R65+S65-b*H65	=-1/Yc_ox*R65-1/Yc_an*S65
=R66+S66-b*H66	=-1/Yc_ox*R66-1/Yc_an*S66
=R67+S67-b*H67	=-1/Yc_ox*R67-1/Yc_an*S67
=R68+S68-b*H68	=-1/Yc_ox*R68-1/Yc_an*S68
=R69+S69-b*H69	=-1/Yc_ox*R69-1/Yc_an*S69
=R70+S70-b*H70	=-1/Yc_ox*R70-1/Yc_an*S70
=R71+S71-b*H71	=-1/Yc_ox*R71-1/Yc_an*S71
=R72+S72-b*H72	=-1/Yc_ox*R72-1/Yc_an*S72

fsa	fEo	fEna
=-((1/Yc_an)/(2.86*Yc_an))*S22	=P22-bO*K22	=Q22-bNO*L22
=-((1/Yc_an)/(2.86*Yc_an))*S23	=P23-bO*K23	=Q23-bNO*L23
=-((1/Yc_an)/(2.86*Yc_an))*S24	=P24-bO*K24	=Q24-bNO*L24
=-((1/Yc_an)/(2.86*Yc_an))*S25	=P25-bO*K25	=Q25-bNO*L25
=-((1/Yc_an)/(2.86*Yc_an))*S26	=P24-bO*K26	=Q26-bNO*L26
=-((1/Yc_an)/(2.86*Yc_an))*S27	=P27-bO*K27	=Q27-bNO*L27
=-((1/Yc_an)/(2.86*Yc_an))*S28	=P28-bO*K28	=Q28-bNO*L28
=-((1/Yc_an)/(2.86*Yc_an))*S29	=P29-bO*K29	=Q29-bNO*L29
=-((1/Yc_an)/(2.86*Yc_an))*S30	=P30-bO*K30	=Q30-bNO*L30
=-((1/Yc_an)/(2.86*Yc_an))*S31	=P31-bO*K31	=Q31-bNO*L31
=-((1/Yc_an)/(2.86*Yc_an))*S32	=P32-bO*K32	=Q32-bNO*L32
=-((1/Yc_an)/(2.86*Yc_an))*S33	=P33-bO*K33	=Q33-bNO*L33
=-((1/Yc_an)/(2.86*Yc_an))*S34	=P34-bO*K34	=Q34-bNO*L34
=-((1/Yc_an)/(2.86*Yc_an))*S35	=P35-bO*K35	=Q35-bNO*L35
=-((1/Yc_an)/(2.86*Yc_an))*S36	=P36-bO*K36	=Q36-bNO*L36
=-((1/Yc_an)/(2.86*Yc_an))*S37	=P37-bO*K37	=Q37-bNO*L37
=-((1/Yc_an)/(2.86*Yc_an))*S38	=P38-bO*K38	=Q38-bNO*L38
=-((1/Yc_an)/(2.86*Yc_an))*S39	=P39-bO*K39	=Q39-bNO*L39
=-((1/Yc_an)/(2.86*Yc_an))*S40	=P40-bO*K40	=Q40-bNO*L40
=-((1/Yc_an)/(2.86*Yc_an))*S41	=P41-bO*K41	=Q41-bNO*L41
=-((1/Yc_an)/(2.86*Yc_an))*S42	=P42-bO*K42	=Q42-bNO*L42
=-((1/Yc_an)/(2.86*Yc_an))*S43	=P43-bO*K43	=Q43-bNO*L43
=-((1/Yc_an)/(2.86*Yc_an))*S44	=P44-bO*K44	=Q44-bNO*L44
=-((1/Yc_an)/(2.86*Yc_an))*S45	=P45-bO*K45	=Q45-bNO*L45
=-((1/Yc_an)/(2.86*Yc_an))*S46	=P46-bO*K46	=Q46-bNO*L46
=-((1/Yc_an)/(2.86*Yc_an))*S47	=P47-bO*K47	=Q47-bNO*L47
=-((1/Yc_an)/(2.86*Yc_an))*S48	=P48-bO*K48	=Q48-bNO*L48
=-((1/Yc_an)/(2.86*Yc_an))*S49	=P49-bO*K49	=Q49-bNO*L49
=-((1/Yc_an)/(2.86*Yc_an))*S50	=P50-bO*K50	=Q50-bNO*L50
=-((1/Yc_an)/(2.86*Yc_an))*S51	=P51-bO*K51	=Q51-bNO*L51
=-((1/Yc_an)/(2.86*Yc_an))*S52	=P52-bO*K52	=Q52-bNO*L52
=-((1/Yc_an)/(2.86*Yc_an))*S53	=P53-bO*K53	=Q53-bNO*L53
=-((1/Yc_an)/(2.86*Yc_an))*S54	=P54-bO*K54	=Q54-bNO*L54
=-((1/Yc_an)/(2.86*Yc_an))*S55	=P55-bO*K55	=Q55-bNO*L55
=-((1/Yc_an)/(2.86*Yc_an))*S56	=P56-bO*K56	=Q56-bNO*L56
=-((1/Yc_an)/(2.86*Yc_an))*S57	=P57-bO*K57	=Q57-bNO*L57
=-((1/Yc_an)/(2.86*Yc_an))*S58	=P58-bO*K58	=Q58-bNO*L58
=-((1/Yc_an)/(2.86*Yc_an))*S59	=P59-bO*K59	=Q59-bNO*L59
=-((1/Yc_an)/(2.86*Yc_an))*S60	=P60-bO*K60	=Q60-bNO*L60
=-((1/Yc_an)/(2.86*Yc_an))*S61	=P61-bO*K61	=Q61-bNO*L61
=-((1/Yc_an)/(2.86*Yc_an))*S62	=P62-bO*K62	=Q62-bNO*L62
=-((1/Yc_an)/(2.86*Yc_an))*S63	=P63-bO*K63	=Q63-bNO*L63
=-((1/Yc_an)/(2.86*Yc_an))*S64	=P64-bO*K64	=Q64-bNO*L64
=-((1/Yc_an)/(2.86*Yc_an))*S65	=P65-bO*K65	=Q65-bNO*L65
=-((1/Yc_an)/(2.86*Yc_an))*S66	=P66-bO*K66	=Q66-bNO*L66
=-((1/Yc_an)/(2.86*Yc_an))*S67	=P67-bO*K67	=Q67-bNO*L67
=-((1/Yc_an)/(2.86*Yc_an))*S68	=P68-bO*K68	=Q68-bNO*L68
=-((1/Yc_an)/(2.86*Yc_an))*S69	=P69-bO*K69	=Q69-bNO*L69
=-((1/Yc_an)/(2.86*Yc_an))*S70	=P70-bO*K70	=Q70-bNO*L70
=-((1/Yc_an)/(2.86*Yc_an))*S71	=P71-bO*K71	=Q71-bNO*L71
=-((1/Yc_an)/(2.86*Yc_an))*S72	=P72-bO*K72	=Q72-bNO*L72

Spreadsheet for preliminary modal file

Parameters	
mumax_ox	0.2592891336021898
mumax_an	0.1094
Ks_ox	242.882571067961
Ks_an	100
Yc_ox	0.9
Yc_an	1.2
Koh	0.129142305751082
Kno	0.129
b	0
aO_1	0
aO_2	0.0058928885296949
aNO_1	0.00009
aNO_2	0.00034803834531973
bO	0.317874132358861
bNO	0.5
Ki	0.5
So	9
D	0.03

Initial conditions	
Xb	=F25
Sa	881.912893060835
Sn	0
Eo	1.67283453186063
Eno	0.000000000016
p	0.1
eo	=Eo/Xb
eno	=Eno/Xb
Run conditions	
eo_max	=(aO_1 + aO_2) / (mumax_ox + bO b)
eno_max	=(aNO_1 + aNO_2) / (mumax_an + bNO b)
n_ox	1.8218888504615
n_anox	7.54427689898704
Dt	0.1
Dt2	0.01
delta	=C40/C39
aerobic SES	=SUM(G25 G1490)
total SES	=SUM(G25 G3890)
anoxic SES	=SUM(G1491 G3890)

t elapsed	abs	mg/L bio	t elapsed
0	0.217	=C25*690*1.42	0
0.133333333244062	0.221	=C26*690*1.42	0.1
1.46666666655801	0.277	=C27*690*1.42	0.2
2.15000000002328	0.293	=C28*690*1.42	0.3
13.9833333333372	0.658	=C29*690*1.42	0.4
19.5999999999534	0.624	=C30*690*1.42	0.5
27.0999999999767	0.753	=C31*690*1.42	0.6
38.8666666666278	0.607	=C32*690*1.42	0.7
52.0499999993302	0.594	=C33*690*1.42	0.8
63.7999999996684	0.578	=C34*690*1.42	0.9
74.7833333333256	0.571	=C35*690*1.42	1
84.8166666666861	0.561	=C36*690*1.42	1.1
112.283333333326	0.57	=C37*690*1.42	1.2
118.983333333302	0.561	=C38*690*1.42	1.3
134.333333333288	0.574	=C39*690*1.42	1.4
137.533333333209	0.101	=C40*690*1.42	1.5
137.733333333337	0.103	=C41*690*1.42	1.6
137.916666666657	0.105	=C42*690*1.42	1.7
138.066666666535	0.106	=C43*690*1.42	1.8
138.266666666663	0.108	=C44*690*1.42	1.9
138.433333333348	0.11	=C45*690*1.42	2
138.849999999977	0.116	=C46*690*1.42	2.1
139.450000000012	0.124	=C47*690*1.42	2.2
139.633333333244	0.127	=C48*690*1.42	2.3
141.416666666628	0.152	=C49*690*1.42	2.4
142.566666666535	0.172	=C50*690*1.42	2.5
144.199999999953	0.207	=C51*690*1.42	2.6
145.416666666657	0.239	=C52*690*1.42	2.7
146.100000000035	0.258	=C53*690*1.42	2.8
157.333333333314	0.812	=C54*690*1.42	2.9
157.699999999906	0.838	=C55*690*1.42	3
158.416666666686	0.856	=C56*690*1.42	3.1
158.866666666581	0.88	=C57*690*1.42	3.2
160.283333333326	0.948	=C58*690*1.42	3.3
160.666666666686	0.942	=C59*690*1.42	3.4
161.383333333244	0.837	=C60*690*1.42	3.5
			3.6
			3.7
			3.8
			3.9
			4
			4.1
			4.2
			4.3
			4.4
			4.5
			4.6
			4.7
			4.8
			4.9
			5

Xh measured	error^2	Xh
=D25	=IF(F25<>"",(F25-H25)^2,"")	=Xh
=D26	=IF(F26<>"",(F26-H26)^2,"")	=H25+Dt*T25
	=IF(F27<>"",(F27-H27)^2,"")	=H26+Dt*T26
	=IF(F28<>"",(F28-H28)^2,"")	=H27+Dt*T27
	=IF(F29<>"",(F29-H29)^2,"")	=H28+Dt*T28
	=IF(F30<>"",(F30-H30)^2,"")	=H29+Dt*T29
	=IF(F31<>"",(F31-H31)^2,"")	=H30+Dt*T30
	=IF(F32<>"",(F32-H32)^2,"")	=H31+Dt*T31
	=IF(F33<>"",(F33-H33)^2,"")	=H32+Dt*T32
	=IF(F34<>"",(F34-H34)^2,"")	=H33+Dt*T33
	=IF(F35<>"",(F35-H35)^2,"")	=H34+Dt*T34
	=IF(F36<>"",(F36-H36)^2,"")	=H35+Dt*T35
	=IF(F37<>"",(F37-H37)^2,"")	=H36+Dt*T36
	=IF(F38<>"",(F38-H38)^2,"")	=H37+Dt*T37
	=IF(F39<>"",(F39-H39)^2,"")	=H38+Dt*T38
=D27	=IF(F40<>"",(F40-H40)^2,"")	=H39+Dt*T39
	=IF(F41<>"",(F41-H41)^2,"")	=H40+Dt*T40
	=IF(F42<>"",(F42-H42)^2,"")	=H41+Dt*T41
	=IF(F43<>"",(F43-H43)^2,"")	=H42+Dt*T42
	=IF(F44<>"",(F44-H44)^2,"")	=H43+Dt*T43
	=IF(F45<>"",(F45-H45)^2,"")	=H44+Dt*T44
	=IF(F46<>"",(F46-H46)^2,"")	=H45+Dt*T45
=D28	=IF(F47<>"",(F47-H47)^2,"")	=H46+Dt*T46
	=IF(F48<>"",(F48-H48)^2,"")	=H47+Dt*T47
	=IF(F49<>"",(F49-H49)^2,"")	=H48+Dt*T48
	=IF(F50<>"",(F50-H50)^2,"")	=H49+Dt*T49
	=IF(F51<>"",(F51-H51)^2,"")	=H50+Dt*T50
	=IF(F52<>"",(F52-H52)^2,"")	=H51+Dt*T51
	=IF(F53<>"",(F53-H53)^2,"")	=H52+Dt*T52
	=IF(F54<>"",(F54-H54)^2,"")	=H53+Dt*T53
	=IF(F55<>"",(F55-H55)^2,"")	=H54+Dt*T54
	=IF(F56<>"",(F56-H56)^2,"")	=H55+Dt*T55
	=IF(F57<>"",(F57-H57)^2,"")	=H56+Dt*T56
	=IF(F58<>"",(F58-H58)^2,"")	=H57+Dt*T57
	=IF(F59<>"",(F59-H59)^2,"")	=H58+Dt*T58
	=IF(F60<>"",(F60-H60)^2,"")	=H59+Dt*T59
	=IF(F61<>"",(F61-H61)^2,"")	=H60+Dt*T60
	=IF(F62<>"",(F62-H62)^2,"")	=H61+Dt*T61
	=IF(F63<>"",(F63-H63)^2,"")	=H62+Dt*T62
	=IF(F64<>"",(F64-H64)^2,"")	=H63+Dt*T63
	=IF(F65<>"",(F65-H65)^2,"")	=H64+Dt*T64
	=IF(F66<>"",(F66-H66)^2,"")	=H65+Dt*T65
	=IF(F67<>"",(F67-H67)^2,"")	=H66+Dt*T66
	=IF(F68<>"",(F68-H68)^2,"")	=H67+Dt*T67
	=IF(F69<>"",(F69-H69)^2,"")	=H68+Dt*T68
	=IF(F70<>"",(F70-H70)^2,"")	=H69+Dt*T69
	=IF(F71<>"",(F71-H71)^2,"")	=H70+Dt*T70
	=IF(F72<>"",(F72-H72)^2,"")	=H71+Dt*T71
	=IF(F73<>"",(F73-H73)^2,"")	=H72+Dt*T72
	=IF(F74<>"",(F74-H74)^2,"")	=H73+Dt*T73
	=IF(F75<>"",(F75-H75)^2,"")	=H74+Dt*T74

Ss	Sn	Eo
=Ss	=Sn	=Eo
=MAX(I25+Dt*U25,0)	=MAX(J25+Dt*V25,0)	=K25+Dt*W25
=MAX(I26+Dt*U26,0)	=MAX(J26+Dt*V26,0)	=K26+Dt*W26
=MAX(I27+Dt*U27,0)	=MAX(J27+Dt*V27,0)	=K27+Dt*W27
=MAX(I28+Dt*U28,0)	=MAX(J28+Dt*V28,0)	=K28+Dt*W28
=MAX(I29+Dt*U29,0)	=MAX(J29+Dt*V29,0)	=K29+Dt*W29
=MAX(I30+Dt*U30,0)	=MAX(J30+Dt*V30,0)	=K30+Dt*W30
=MAX(I31+Dt*U31,0)	=MAX(J31+Dt*V31,0)	=K31+Dt*W31
=MAX(I32+Dt*U32,0)	=MAX(J32+Dt*V32,0)	=K32+Dt*W32
=MAX(I33+Dt*U33,0)	=MAX(J33+Dt*V33,0)	=K33+Dt*W33
=MAX(I34+Dt*U34,0)	=MAX(J34+Dt*V34,0)	=K34+Dt*W34
=MAX(I35+Dt*U35,0)	=MAX(J35+Dt*V35,0)	=K35+Dt*W35
=MAX(I36+Dt*U36,0)	=MAX(J36+Dt*V36,0)	=K36+Dt*W36
=MAX(I37+Dt*U37,0)	=MAX(J37+Dt*V37,0)	=K37+Dt*W37
=MAX(I38+Dt*U38,0)	=MAX(J38+Dt*V38,0)	=K38+Dt*W38
=MAX(I39+Dt*U39,0)	=MAX(J39+Dt*V39,0)	=K39+Dt*W39
=MAX(I40+Dt*U40,0)	=MAX(J40+Dt*V40,0)	=K40+Dt*W40
=MAX(I41+Dt*U41,0)	=MAX(J41+Dt*V41,0)	=K41+Dt*W41
=MAX(I42+Dt*U42,0)	=MAX(J42+Dt*V42,0)	=K42+Dt*W42
=MAX(I43+Dt*U43,0)	=MAX(J43+Dt*V43,0)	=K43+Dt*W43
=MAX(I44+Dt*U44,0)	=MAX(J44+Dt*V44,0)	=K44+Dt*W44
=MAX(I45+Dt*U45,0)	=MAX(J45+Dt*V45,0)	=K45+Dt*W45
=MAX(I46+Dt*U46,0)	=MAX(J46+Dt*V46,0)	=K46+Dt*W46
=MAX(I47+Dt*U47,0)	=MAX(J47+Dt*V47,0)	=K47+Dt*W47
=MAX(I48+Dt*U48,0)	=MAX(J48+Dt*V48,0)	=K48+Dt*W48
=MAX(I49+Dt*U49,0)	=MAX(J49+Dt*V49,0)	=K49+Dt*W49
=MAX(I50+Dt*U50,0)	=MAX(J50+Dt*V50,0)	=K50+Dt*W50
=MAX(I51+Dt*U51,0)	=MAX(J51+Dt*V51,0)	=K51+Dt*W51
=MAX(I52+Dt*U52,0)	=MAX(J52+Dt*V52,0)	=K52+Dt*W52
=MAX(I53+Dt*U53,0)	=MAX(J53+Dt*V53,0)	=K53+Dt*W53
=MAX(I54+Dt*U54,0)	=MAX(J54+Dt*V54,0)	=K54+Dt*W54
=MAX(I55+Dt*U55,0)	=MAX(J55+Dt*V55,0)	=K55+Dt*W55
=MAX(I56+Dt*U56,0)	=MAX(J56+Dt*V56,0)	=K56+Dt*W56
=MAX(I57+Dt*U57,0)	=MAX(J57+Dt*V57,0)	=K57+Dt*W57
=MAX(I58+Dt*U58,0)	=MAX(J58+Dt*V58,0)	=K58+Dt*W58
=MAX(I59+Dt*U59,0)	=MAX(J59+Dt*V59,0)	=K59+Dt*W59
=MAX(I60+Dt*U60,0)	=MAX(J60+Dt*V60,0)	=K60+Dt*W60
=MAX(I61+Dt*U61,0)	=MAX(J61+Dt*V61,0)	=K61+Dt*W61
=MAX(I62+Dt*U62,0)	=MAX(J62+Dt*V62,0)	=K62+Dt*W62
=MAX(I63+Dt*U63,0)	=MAX(J63+Dt*V63,0)	=K63+Dt*W63
=MAX(I64+Dt*U64,0)	=MAX(J64+Dt*V64,0)	=K64+Dt*W64
=MAX(I65+Dt*U65,0)	=MAX(J65+Dt*V65,0)	=K65+Dt*W65
=MAX(I66+Dt*U66,0)	=MAX(J66+Dt*V66,0)	=K66+Dt*W66
=MAX(I67+Dt*U67,0)	=MAX(J67+Dt*V67,0)	=K67+Dt*W67
=MAX(I68+Dt*U68,0)	=MAX(J68+Dt*V68,0)	=K68+Dt*W68
=MAX(I69+Dt*U69,0)	=MAX(J69+Dt*V69,0)	=K69+Dt*W69
=MAX(I70+Dt*U70,0)	=MAX(J70+Dt*V70,0)	=K70+Dt*W70
=MAX(I71+Dt*U71,0)	=MAX(J71+Dt*V71,0)	=K71+Dt*W71
=MAX(I72+Dt*U72,0)	=MAX(J72+Dt*V72,0)	=K72+Dt*W72
=MAX(I73+Dt*U73,0)	=MAX(J73+Dt*V73,0)	=K73+Dt*W73
=MAX(I74+Dt*U74,0)	=MAX(J74+Dt*V74,0)	=K74+Dt*W74

Eno	eo	eno	So
=Eno	=K25/H25	=L25/H25	8
=L25+Dt*X25	=K26/H26	=L26/H26	8
=L26+Dt*X26	=K27/H27	=L27/H27	8
=L27+Dt*X27	=K28/H28	=L28/H28	8
=L28+Dt*X28	=K29/H29	=L29/H29	8
=L29+Dt*X29	=K30/H30	=L30/H30	8
=L30+Dt*X30	=K31/H31	=L31/H31	8
=L31+Dt*X31	=K32/H32	=L32/H32	8
=L32+Dt*X32	=K33/H33	=L33/H33	8
=L33+Dt*X33	=K34/H34	=L34/H34	8
=L34+Dt*X34	=K35/H35	=L35/H35	8
=L35+Dt*X35	=K36/H36	=L36/H36	8
=L36+Dt*X36	=K37/H37	=L37/H37	8
=L37+Dt*X37	=K38/H38	=L38/H38	8
=L38+Dt*X38	=K39/H39	=L39/H39	8
=L39+Dt*X39	=K40/H40	=L40/H40	8
=L40+Dt*X40	=K41/H41	=L41/H41	8
=L41+Dt*X41	=K42/H42	=L42/H42	8
=L42+Dt*X42	=K43/H43	=L43/H43	8
=L43+Dt*X43	=K44/H44	=L44/H44	8
=L44+Dt*X44	=K45/H45	=L45/H45	8
=L45+Dt*X45	=K46/H46	=L46/H46	8
=L46+Dt*X46	=K47/H47	=L47/H47	8
=L47+Dt*X47	=K48/H48	=L48/H48	8
=L48+Dt*X48	=K49/H49	=L49/H49	8
=L49+Dt*X49	=K50/H50	=L50/H50	8
=L50+Dt*X50	=K51/H51	=L51/H51	8
=L51+Dt*X51	=K52/H52	=L52/H52	8
=L52+Dt*X52	=K53/H53	=L53/H53	8
=L53+Dt*X53	=K54/H54	=L54/H54	8
=L54+Dt*X54	=K55/H55	=L55/H55	8
=L55+Dt*X55	=K56/H56	=L56/H56	8
=L56+Dt*X56	=K57/H57	=L57/H57	8
=L57+Dt*X57	=K58/H58	=L58/H58	8
=L58+Dt*X58	=K59/H59	=L59/H59	8
=L59+Dt*X59	=K60/H60	=L60/H60	8
=L60+Dt*X60	=K61/H61	=L61/H61	8
=L61+Dt*X61	=K62/H62	=L62/H62	8
=L62+Dt*X62	=K63/H63	=L63/H63	8
=L63+Dt*X63	=K64/H64	=L64/H64	8
=L64+Dt*X64	=K65/H65	=L65/H65	8
=L65+Dt*X65	=K66/H66	=L66/H66	8
=L66+Dt*X66	=K67/H67	=L67/H67	8
=L67+Dt*X67	=K68/H68	=L68/H68	8
=L68+Dt*X68	=K69/H69	=L69/H69	8
=L69+Dt*X69	=K70/H70	=L70/H70	8
=L70+Dt*X70	=K71/H71	=L71/H71	8
=L71+Dt*X71	=K72/H72	=L72/H72	8
=L72+Dt*X72	=K73/H73	=L73/H73	8
=L73+Dt*X73	=K74/H74	=L74/H74	8
=L74+Dt*X74	=K75/H75	=L75/H75	8

rate Eo

```
=(aO_1+aO_2*M25/eo_max)*O25/(Koh+O25)*I25/(Ks_ox+I25)*H25
=(aO_1+aO_2*M26/eo_max)*O26/(Koh+O26)*I26/(Ks_ox+I26)*H26
=(aO_1+aO_2*M27/eo_max)*O27/(Koh+O27)*I27/(Ks_ox+I27)*H27
=(aO_1+aO_2*M28/eo_max)*O28/(Koh+O28)*I28/(Ks_ox+I28)*H28
=(aO_1+aO_2*M29/eo_max)*O29/(Koh+O29)*I29/(Ks_ox+I29)*H29
=(aO_1+aO_2*M30/eo_max)*O30/(Koh+O30)*I30/(Ks_ox+I30)*H30
=(aO_1+aO_2*M31/eo_max)*O31/(Koh+O31)*I31/(Ks_ox+I31)*H31
=(aO_1+aO_2*M32/eo_max)*O32/(Koh+O32)*I32/(Ks_ox+I32)*H32
=(aO_1+aO_2*M33/eo_max)*O33/(Koh+O33)*I33/(Ks_ox+I33)*H33
=(aO_1+aO_2*M34/eo_max)*O34/(Koh+O34)*I34/(Ks_ox+I34)*H34
=(aO_1+aO_2*M35/eo_max)*O35/(Koh+O35)*I35/(Ks_ox+I35)*H35
=(aO_1+aO_2*M36/eo_max)*O36/(Koh+O36)*I36/(Ks_ox+I36)*H36
=(aO_1+aO_2*M37/eo_max)*O37/(Koh+O37)*I37/(Ks_ox+I37)*H37
=(aO_1+aO_2*M38/eo_max)*O38/(Koh+O38)*I38/(Ks_ox+I38)*H38
=(aO_1+aO_2*M39/eo_max)*O39/(Koh+O39)*I39/(Ks_ox+I39)*H39
=(aO_1+aO_2*M40/eo_max)*O40/(Koh+O40)*I40/(Ks_ox+I40)*H40
=(aO_1+aO_2*M41/eo_max)*O41/(Koh+O41)*I41/(Ks_ox+I41)*H41
=(aO_1+aO_2*M42/eo_max)*O42/(Koh+O42)*I42/(Ks_ox+I42)*H42
=(aO_1+aO_2*M43/eo_max)*O43/(Koh+O43)*I43/(Ks_ox+I43)*H43
=(aO_1+aO_2*M44/eo_max)*O44/(Koh+O44)*I44/(Ks_ox+I44)*H44
=(aO_1+aO_2*M45/eo_max)*O45/(Koh+O45)*I45/(Ks_ox+I45)*H45
=(aO_1+aO_2*M46/eo_max)*O46/(Koh+O46)*I46/(Ks_ox+I46)*H46
=(aO_1+aO_2*M47/eo_max)*O47/(Koh+O47)*I47/(Ks_ox+I47)*H47
=(aO_1+aO_2*M48/eo_max)*O48/(Koh+O48)*I48/(Ks_ox+I48)*H48
=(aO_1+aO_2*M49/eo_max)*O49/(Koh+O49)*I49/(Ks_ox+I49)*H49
=(aO_1+aO_2*M50/eo_max)*O50/(Koh+O50)*I50/(Ks_ox+I50)*H50
=(aO_1+aO_2*M51/eo_max)*O51/(Koh+O51)*I51/(Ks_ox+I51)*H51
=(aO_1+aO_2*M52/eo_max)*O52/(Koh+O52)*I52/(Ks_ox+I52)*H52
=(aO_1+aO_2*M53/eo_max)*O53/(Koh+O53)*I53/(Ks_ox+I53)*H53
=(aO_1+aO_2*M54/eo_max)*O54/(Koh+O54)*I54/(Ks_ox+I54)*H54
=(aO_1+aO_2*M55/eo_max)*O55/(Koh+O55)*I55/(Ks_ox+I55)*H55
=(aO_1+aO_2*M56/eo_max)*O56/(Koh+O56)*I56/(Ks_ox+I56)*H56
=(aO_1+aO_2*M57/eo_max)*O57/(Koh+O57)*I57/(Ks_ox+I57)*H57
=(aO_1+aO_2*M58/eo_max)*O58/(Koh+O58)*I58/(Ks_ox+I58)*H58
=(aO_1+aO_2*M59/eo_max)*O59/(Koh+O59)*I59/(Ks_ox+I59)*H59
=(aO_1+aO_2*M60/eo_max)*O60/(Koh+O60)*I60/(Ks_ox+I60)*H60
=(aO_1+aO_2*M61/eo_max)*O61/(Koh+O61)*I61/(Ks_ox+I61)*H61
=(aO_1+aO_2*M62/eo_max)*O62/(Koh+O62)*I62/(Ks_ox+I62)*H62
=(aO_1+aO_2*M63/eo_max)*O63/(Koh+O63)*I63/(Ks_ox+I63)*H63
=(aO_1+aO_2*M64/eo_max)*O64/(Koh+O64)*I64/(Ks_ox+I64)*H64
=(aO_1+aO_2*M65/eo_max)*O65/(Koh+O65)*I65/(Ks_ox+I65)*H65
=(aO_1+aO_2*M66/eo_max)*O66/(Koh+O66)*I66/(Ks_ox+I66)*H66
=(aO_1+aO_2*M67/eo_max)*O67/(Koh+O67)*I67/(Ks_ox+I67)*H67
=(aO_1+aO_2*M68/eo_max)*O68/(Koh+O68)*I68/(Ks_ox+I68)*H68
=(aO_1+aO_2*M69/eo_max)*O69/(Koh+O69)*I69/(Ks_ox+I69)*H69
=(aO_1+aO_2*M70/eo_max)*O70/(Koh+O70)*I70/(Ks_ox+I70)*H70
=(aO_1+aO_2*M71/eo_max)*O71/(Koh+O71)*I71/(Ks_ox+I71)*H71
=(aO_1+aO_2*M72/eo_max)*O72/(Koh+O72)*I72/(Ks_ox+I72)*H72
=(aO_1+aO_2*M73/eo_max)*O73/(Koh+O73)*I73/(Ks_ox+I73)*H73
=(aO_1+aO_2*M74/eo_max)*O74/(Koh+O74)*I74/(Ks_ox+I74)*H74
=(aO_1+aO_2*M75/eo_max)*O75/(Koh+O75)*I75/(Ks_ox+I75)*H75
```

```
rate Eno
=(aNO_1+aNO_2*N25/eno_max)*J25/(Kno+J25)*I25/(Ks_an+I25)*H25*Ki/(Ki+O25)
=(aNO_1+aNO_2*N26/eno_max)*J26/(Kno+J26)*I26/(Ks_an+I26)*H26*Ki/(Ki+O26)
=(aNO_1+aNO_2*N27/eno_max)*J27/(Kno+J27)*I27/(Ks_an+I27)*H27*Ki/(Ki+O27)
=(aNO_1+aNO_2*N28/eno_max)*J28/(Kno+J28)*I28/(Ks_an+I28)*H28*Ki/(Ki+O28)
=(aNO_1+aNO_2*N29/eno_max)*J29/(Kno+J29)*I29/(Ks_an+I29)*H29*Ki/(Ki+O29)
=(aNO_1+aNO_2*N30/eno_max)*J30/(Kno+J30)*I30/(Ks_an+I30)*H30*Ki/(Ki+O30)
=(aNO_1+aNO_2*N31/eno_max)*J31/(Kno+J31)*I31/(Ks_an+I31)*H31*Ki/(Ki+O31)
=(aNO_1+aNO_2*N32/eno_max)*J32/(Kno+J32)*I32/(Ks_an+I32)*H32*Ki/(Ki+O32)
=(aNO_1+aNO_2*N33/eno_max)*J33/(Kno+J33)*I33/(Ks_an+I33)*H33*Ki/(Ki+O33)
=(aNO_1+aNO_2*N34/eno_max)*J34/(Kno+J34)*I34/(Ks_an+I34)*H34*Ki/(Ki+O34)
=(aNO_1+aNO_2*N35/eno_max)*J35/(Kno+J35)*I35/(Ks_an+I35)*H35*Ki/(Ki+O35)
=(aNO_1+aNO_2*N36/eno_max)*J36/(Kno+J36)*I36/(Ks_an+I36)*H36*Ki/(Ki+O36)
=(aNO_1+aNO_2*N37/eno_max)*J37/(Kno+J37)*I37/(Ks_an+I37)*H37*Ki/(Ki+O37)
=(aNO_1+aNO_2*N38/eno_max)*J38/(Kno+J38)*I38/(Ks_an+I38)*H38*Ki/(Ki+O38)
=(aNO_1+aNO_2*N39/eno_max)*J39/(Kno+J39)*I39/(Ks_an+I39)*H39*Ki/(Ki+O39)
=(aNO_1+aNO_2*N40/eno_max)*J40/(Kno+J40)*I40/(Ks_an+I40)*H40*Ki/(Ki+O40)
=(aNO_1+aNO_2*N41/eno_max)*J41/(Kno+J41)*I41/(Ks_an+I41)*H41*Ki/(Ki+O41)
=(aNO_1+aNO_2*N42/eno_max)*J42/(Kno+J42)*I42/(Ks_an+I42)*H42*Ki/(Ki+O42)
=(aNO_1+aNO_2*N43/eno_max)*J43/(Kno+J43)*I43/(Ks_an+I43)*H43*Ki/(Ki+O43)
=(aNO_1+aNO_2*N44/eno_max)*J44/(Kno+J44)*I44/(Ks_an+I44)*H44*Ki/(Ki+O44)
=(aNO_1+aNO_2*N45/eno_max)*J45/(Kno+J45)*I45/(Ks_an+I45)*H45*Ki/(Ki+O45)
=(aNO_1+aNO_2*N46/eno_max)*J46/(Kno+J46)*I46/(Ks_an+I46)*H46*Ki/(Ki+O46)
=(aNO_1+aNO_2*N47/eno_max)*J47/(Kno+J47)*I47/(Ks_an+I47)*H47*Ki/(Ki+O47)
=(aNO_1+aNO_2*N48/eno_max)*J48/(Kno+J48)*I48/(Ks_an+I48)*H48*Ki/(Ki+O48)
=(aNO_1+aNO_2*N49/eno_max)*J49/(Kno+J49)*I49/(Ks_an+I49)*H49*Ki/(Ki+O49)
=(aNO_1+aNO_2*N50/eno_max)*J50/(Kno+J50)*I50/(Ks_an+I50)*H50*Ki/(Ki+O50)
=(aNO_1+aNO_2*N51/eno_max)*J51/(Kno+J51)*I51/(Ks_an+I51)*H51*Ki/(Ki+O51)
=(aNO_1+aNO_2*N52/eno_max)*J52/(Kno+J52)*I52/(Ks_an+I52)*H52*Ki/(Ki+O52)
=(aNO_1+aNO_2*N53/eno_max)*J53/(Kno+J53)*I53/(Ks_an+I53)*H53*Ki/(Ki+O53)
=(aNO_1+aNO_2*N54/eno_max)*J54/(Kno+J54)*I54/(Ks_an+I54)*H54*Ki/(Ki+O54)
=(aNO_1+aNO_2*N55/eno_max)*J55/(Kno+J55)*I55/(Ks_an+I55)*H55*Ki/(Ki+O55)
=(aNO_1+aNO_2*N56/eno_max)*J56/(Kno+J56)*I56/(Ks_an+I56)*H56*Ki/(Ki+O56)
=(aNO_1+aNO_2*N57/eno_max)*J57/(Kno+J57)*I57/(Ks_an+I57)*H57*Ki/(Ki+O57)
=(aNO_1+aNO_2*N58/eno_max)*J58/(Kno+J58)*I58/(Ks_an+I58)*H58*Ki/(Ki+O58)
=(aNO_1+aNO_2*N59/eno_max)*J59/(Kno+J59)*I59/(Ks_an+I59)*H59*Ki/(Ki+O59)
=(aNO_1+aNO_2*N60/eno_max)*J60/(Kno+J60)*I60/(Ks_an+I60)*H60*Ki/(Ki+O60)
=(aNO_1+aNO_2*N61/eno_max)*J61/(Kno+J61)*I61/(Ks_an+I61)*H61*Ki/(Ki+O61)
=(aNO_1+aNO_2*N62/eno_max)*J62/(Kno+J62)*I62/(Ks_an+I62)*H62*Ki/(Ki+O62)
=(aNO_1+aNO_2*N63/eno_max)*J63/(Kno+J63)*I63/(Ks_an+I63)*H63*Ki/(Ki+O63)
=(aNO_1+aNO_2*N64/eno_max)*J64/(Kno+J64)*I64/(Ks_an+I64)*H64*Ki/(Ki+O64)
=(aNO_1+aNO_2*N65/eno_max)*J65/(Kno+J65)*I65/(Ks_an+I65)*H65*Ki/(Ki+O65)
=(aNO_1+aNO_2*N66/eno_max)*J66/(Kno+J66)*I66/(Ks_an+I66)*H66*Ki/(Ki+O66)
=(aNO_1+aNO_2*N67/eno_max)*J67/(Kno+J67)*I67/(Ks_an+I67)*H67*Ki/(Ki+O67)
=(aNO_1+aNO_2*N68/eno_max)*J68/(Kno+J68)*I68/(Ks_an+I68)*H68*Ki/(Ki+O68)
=(aNO_1+aNO_2*N69/eno_max)*J69/(Kno+J69)*I69/(Ks_an+I69)*H69*Ki/(Ki+O69)
=(aNO_1+aNO_2*N70/eno_max)*J70/(Kno+J70)*I70/(Ks_an+I70)*H70*Ki/(Ki+O70)
=(aNO_1+aNO_2*N71/eno_max)*J71/(Kno+J71)*I71/(Ks_an+I71)*H71*Ki/(Ki+O71)
=(aNO_1+aNO_2*N72/eno_max)*J72/(Kno+J72)*I72/(Ks_an+I72)*H72*Ki/(Ki+O72)
=(aNO_1+aNO_2*N73/eno_max)*J73/(Kno+J73)*I73/(Ks_an+I73)*H73*Ki/(Ki+O73)
=(aNO_1+aNO_2*N74/eno_max)*J74/(Kno+J74)*I74/(Ks_an+I74)*H74*Ki/(Ki+O74)
=(aNO_1+aNO_2*N75/eno_max)*J75/(Kno+J75)*I75/(Ks_an+I75)*H75*Ki/(Ki+O75)
```

rate aer

```
=mumax_ox*((M25/eo_max)^n_ox)*O25/(Koh+O25)*I25/(Ks_ox+I25)*H25
=mumax_ox*((M26/eo_max)^n_ox)*O26/(Koh+O26)*I26/(Ks_ox+I26)*H26
=mumax_ox*((M27/eo_max)^n_ox)*O27/(Koh+O27)*I27/(Ks_ox+I27)*H27
=mumax_ox*((M28/eo_max)^n_ox)*O28/(Koh+O28)*I28/(Ks_ox+I28)*H28
=mumax_ox*((M29/eo_max)^n_ox)*O29/(Koh+O29)*I29/(Ks_ox+I29)*H29
=mumax_ox*((M30/eo_max)^n_ox)*O30/(Koh+O30)*I30/(Ks_ox+I30)*H30
=mumax_ox*((M31/eo_max)^n_ox)*O31/(Koh+O31)*I31/(Ks_ox+I31)*H31
=mumax_ox*((M32/eo_max)^n_ox)*O32/(Koh+O32)*I32/(Ks_ox+I32)*H32
=mumax_ox*((M33/eo_max)^n_ox)*O33/(Koh+O33)*I33/(Ks_ox+I33)*H33
=mumax_ox*((M34/eo_max)^n_ox)*O34/(Koh+O34)*I34/(Ks_ox+I34)*H34
=mumax_ox*((M35/eo_max)^n_ox)*O35/(Koh+O35)*I35/(Ks_ox+I35)*H35
=mumax_ox*((M36/eo_max)^n_ox)*O36/(Koh+O36)*I36/(Ks_ox+I36)*H36
=mumax_ox*((M37/eo_max)^n_ox)*O37/(Koh+O37)*I37/(Ks_ox+I37)*H37
=mumax_ox*((M38/eo_max)^n_ox)*O38/(Koh+O38)*I38/(Ks_ox+I38)*H38
=mumax_ox*((M39/eo_max)^n_ox)*O39/(Koh+O39)*I39/(Ks_ox+I39)*H39
=mumax_ox*((M40/eo_max)^n_ox)*O40/(Koh+O40)*I40/(Ks_ox+I40)*H40
=mumax_ox*((M41/eo_max)^n_ox)*O41/(Koh+O41)*I41/(Ks_ox+I41)*H41
=mumax_ox*((M42/eo_max)^n_ox)*O42/(Koh+O42)*I42/(Ks_ox+I42)*H42
=mumax_ox*((M43/eo_max)^n_ox)*O43/(Koh+O43)*I43/(Ks_ox+I43)*H43
=mumax_ox*((M44/eo_max)^n_ox)*O44/(Koh+O44)*I44/(Ks_ox+I44)*H44
=mumax_ox*((M45/eo_max)^n_ox)*O45/(Koh+O45)*I45/(Ks_ox+I45)*H45
=mumax_ox*((M46/eo_max)^n_ox)*O46/(Koh+O46)*I46/(Ks_ox+I46)*H46
=mumax_ox*((M47/eo_max)^n_ox)*O47/(Koh+O47)*I47/(Ks_ox+I47)*H47
=mumax_ox*((M48/eo_max)^n_ox)*O48/(Koh+O48)*I48/(Ks_ox+I48)*H48
=mumax_ox*((M49/eo_max)^n_ox)*O49/(Koh+O49)*I49/(Ks_ox+I49)*H49
=mumax_ox*((M50/eo_max)^n_ox)*O50/(Koh+O50)*I50/(Ks_ox+I50)*H50
=mumax_ox*((M51/eo_max)^n_ox)*O51/(Koh+O51)*I51/(Ks_ox+I51)*H51
=mumax_ox*((M52/eo_max)^n_ox)*O52/(Koh+O52)*I52/(Ks_ox+I52)*H52
=mumax_ox*((M53/eo_max)^n_ox)*O53/(Koh+O53)*I53/(Ks_ox+I53)*H53
=mumax_ox*((M54/eo_max)^n_ox)*O54/(Koh+O54)*I54/(Ks_ox+I54)*H54
=mumax_ox*((M55/eo_max)^n_ox)*O55/(Koh+O55)*I55/(Ks_ox+I55)*H55
=mumax_ox*((M56/eo_max)^n_ox)*O56/(Koh+O56)*I56/(Ks_ox+I56)*H56
=mumax_ox*((M57/eo_max)^n_ox)*O57/(Koh+O57)*I57/(Ks_ox+I57)*H57
=mumax_ox*((M58/eo_max)^n_ox)*O58/(Koh+O58)*I58/(Ks_ox+I58)*H58
=mumax_ox*((M59/eo_max)^n_ox)*O59/(Koh+O59)*I59/(Ks_ox+I59)*H59
=mumax_ox*((M60/eo_max)^n_ox)*O60/(Koh+O60)*I60/(Ks_ox+I60)*H60
=mumax_ox*((M61/eo_max)^n_ox)*O61/(Koh+O61)*I61/(Ks_ox+I61)*H61
=mumax_ox*((M62/eo_max)^n_ox)*O62/(Koh+O62)*I62/(Ks_ox+I62)*H62
=mumax_ox*((M63/eo_max)^n_ox)*O63/(Koh+O63)*I63/(Ks_ox+I63)*H63
=mumax_ox*((M64/eo_max)^n_ox)*O64/(Koh+O64)*I64/(Ks_ox+I64)*H64
=mumax_ox*((M65/eo_max)^n_ox)*O65/(Koh+O65)*I65/(Ks_ox+I65)*H65
=mumax_ox*((M66/eo_max)^n_ox)*O66/(Koh+O66)*I66/(Ks_ox+I66)*H66
=mumax_ox*((M67/eo_max)^n_ox)*O67/(Koh+O67)*I67/(Ks_ox+I67)*H67
=mumax_ox*((M68/eo_max)^n_ox)*O68/(Koh+O68)*I68/(Ks_ox+I68)*H68
=mumax_ox*((M69/eo_max)^n_ox)*O69/(Koh+O69)*I69/(Ks_ox+I69)*H69
=mumax_ox*((M70/eo_max)^n_ox)*O70/(Koh+O70)*I70/(Ks_ox+I70)*H70
=mumax_ox*((M71/eo_max)^n_ox)*O71/(Koh+O71)*I71/(Ks_ox+I71)*H71
=mumax_ox*((M72/eo_max)^n_ox)*O72/(Koh+O72)*I72/(Ks_ox+I72)*H72
=mumax_ox*((M73/eo_max)^n_ox)*O73/(Koh+O73)*I73/(Ks_ox+I73)*H73
=mumax_ox*((M74/eo_max)^n_ox)*O74/(Koh+O74)*I74/(Ks_ox+I74)*H74
=mumax_ox*((M75/eo_max)^n_ox)*O75/(Koh+O75)*I75/(Ks_ox+I75)*H75
```

rate anox

```
=(p+(N25/eno_max)^n_anox*mumax_an)*Ki/(Ki+O25)*J25/(Kno+J25)*I25/(Ks_an+I25)*H25
=(p+(N26/eno_max)^n_anox*mumax_an)*Ki/(Ki+O26)*J26/(Kno+J26)*I26/(Ks_an+I26)*H26
=(p+(N27/eno_max)^n_anox*mumax_an)*Ki/(Ki+O27)*J27/(Kno+J27)*I27/(Ks_an+I27)*H27
=(p+(N28/eno_max)^n_anox*mumax_an)*Ki/(Ki+O28)*J28/(Kno+J28)*I28/(Ks_an+I28)*H28
=(p+(N29/eno_max)^n_anox*mumax_an)*Ki/(Ki+O29)*J29/(Kno+J29)*I29/(Ks_an+I29)*H29
=(p+(N30/eno_max)^n_anox*mumax_an)*Ki/(Ki+O30)*J30/(Kno+J30)*I30/(Ks_an+I30)*H30
=(p+(N31/eno_max)^n_anox*mumax_an)*Ki/(Ki+O31)*J31/(Kno+J31)*I31/(Ks_an+I31)*H31
=(p+(N32/eno_max)^n_anox*mumax_an)*Ki/(Ki+O32)*J32/(Kno+J32)*I32/(Ks_an+I32)*H32
=(p+(N33/eno_max)^n_anox*mumax_an)*Ki/(Ki+O33)*J33/(Kno+J33)*I33/(Ks_an+I33)*H33
=(p+(N34/eno_max)^n_anox*mumax_an)*Ki/(Ki+O34)*J34/(Kno+J34)*I34/(Ks_an+I34)*H34
=(p+(N35/eno_max)^n_anox*mumax_an)*Ki/(Ki+O35)*J35/(Kno+J35)*I35/(Ks_an+I35)*H35
=(p+(N36/eno_max)^n_anox*mumax_an)*Ki/(Ki+O36)*J36/(Kno+J36)*I36/(Ks_an+I36)*H36
=(p+(N37/eno_max)^n_anox*mumax_an)*Ki/(Ki+O37)*J37/(Kno+J37)*I37/(Ks_an+I37)*H37
=(p+(N38/eno_max)^n_anox*mumax_an)*Ki/(Ki+O38)*J38/(Kno+J38)*I38/(Ks_an+I38)*H38
=(p+(N39/eno_max)^n_anox*mumax_an)*Ki/(Ki+O39)*J39/(Kno+J39)*I39/(Ks_an+I39)*H39
=(p+(N40/eno_max)^n_anox*mumax_an)*Ki/(Ki+O40)*J40/(Kno+J40)*I40/(Ks_an+I40)*H40
=(p+(N41/eno_max)^n_anox*mumax_an)*Ki/(Ki+O41)*J41/(Kno+J41)*I41/(Ks_an+I41)*H41
=(p+(N42/eno_max)^n_anox*mumax_an)*Ki/(Ki+O42)*J42/(Kno+J42)*I42/(Ks_an+I42)*H42
=(p+(N43/eno_max)^n_anox*mumax_an)*Ki/(Ki+O43)*J43/(Kno+J43)*I43/(Ks_an+I43)*H43
=(p+(N44/eno_max)^n_anox*mumax_an)*Ki/(Ki+O44)*J44/(Kno+J44)*I44/(Ks_an+I44)*H44
=(p+(N45/eno_max)^n_anox*mumax_an)*Ki/(Ki+O45)*J45/(Kno+J45)*I45/(Ks_an+I45)*H45
=(p+(N46/eno_max)^n_anox*mumax_an)*Ki/(Ki+O46)*J46/(Kno+J46)*I46/(Ks_an+I46)*H46
=(p+(N47/eno_max)^n_anox*mumax_an)*Ki/(Ki+O47)*J47/(Kno+J47)*I47/(Ks_an+I47)*H47
=(p+(N48/eno_max)^n_anox*mumax_an)*Ki/(Ki+O48)*J48/(Kno+J48)*I48/(Ks_an+I48)*H48
=(p+(N49/eno_max)^n_anox*mumax_an)*Ki/(Ki+O49)*J49/(Kno+J49)*I49/(Ks_an+I49)*H49
=(p+(N50/eno_max)^n_anox*mumax_an)*Ki/(Ki+O50)*J50/(Kno+J50)*I50/(Ks_an+I50)*H50
=(p+(N51/eno_max)^n_anox*mumax_an)*Ki/(Ki+O51)*J51/(Kno+J51)*I51/(Ks_an+I51)*H51
=(p+(N52/eno_max)^n_anox*mumax_an)*Ki/(Ki+O52)*J52/(Kno+J52)*I52/(Ks_an+I52)*H52
=(p+(N53/eno_max)^n_anox*mumax_an)*Ki/(Ki+O53)*J53/(Kno+J53)*I53/(Ks_an+I53)*H53
=(p+(N54/eno_max)^n_anox*mumax_an)*Ki/(Ki+O54)*J54/(Kno+J54)*I54/(Ks_an+I54)*H54
=(p+(N55/eno_max)^n_anox*mumax_an)*Ki/(Ki+O55)*J55/(Kno+J55)*I55/(Ks_an+I55)*H55
=(p+(N56/eno_max)^n_anox*mumax_an)*Ki/(Ki+O56)*J56/(Kno+J56)*I56/(Ks_an+I56)*H56
=(p+(N57/eno_max)^n_anox*mumax_an)*Ki/(Ki+O57)*J57/(Kno+J57)*I57/(Ks_an+I57)*H57
=(p+(N58/eno_max)^n_anox*mumax_an)*Ki/(Ki+O58)*J58/(Kno+J58)*I58/(Ks_an+I58)*H58
=(p+(N59/eno_max)^n_anox*mumax_an)*Ki/(Ki+O59)*J59/(Kno+J59)*I59/(Ks_an+I59)*H59
=(p+(N60/eno_max)^n_anox*mumax_an)*Ki/(Ki+O60)*J60/(Kno+J60)*I60/(Ks_an+I60)*H60
=(p+(N61/eno_max)^n_anox*mumax_an)*Ki/(Ki+O61)*J61/(Kno+J61)*I61/(Ks_an+I61)*H61
=(p+(N62/eno_max)^n_anox*mumax_an)*Ki/(Ki+O62)*J62/(Kno+J62)*I62/(Ks_an+I62)*H62
=(p+(N63/eno_max)^n_anox*mumax_an)*Ki/(Ki+O63)*J63/(Kno+J63)*I63/(Ks_an+I63)*H63
=(p+(N64/eno_max)^n_anox*mumax_an)*Ki/(Ki+O64)*J64/(Kno+J64)*I64/(Ks_an+I64)*H64
=(p+(N65/eno_max)^n_anox*mumax_an)*Ki/(Ki+O65)*J65/(Kno+J65)*I65/(Ks_an+I65)*H65
=(p+(N66/eno_max)^n_anox*mumax_an)*Ki/(Ki+O66)*J66/(Kno+J66)*I66/(Ks_an+I66)*H66
=(p+(N67/eno_max)^n_anox*mumax_an)*Ki/(Ki+O67)*J67/(Kno+J67)*I67/(Ks_an+I67)*H67
=(p+(N68/eno_max)^n_anox*mumax_an)*Ki/(Ki+O68)*J68/(Kno+J68)*I68/(Ks_an+I68)*H68
=(p+(N69/eno_max)^n_anox*mumax_an)*Ki/(Ki+O69)*J69/(Kno+J69)*I69/(Ks_an+I69)*H69
=(p+(N70/eno_max)^n_anox*mumax_an)*Ki/(Ki+O70)*J70/(Kno+J70)*I70/(Ks_an+I70)*H70
=(p+(N71/eno_max)^n_anox*mumax_an)*Ki/(Ki+O71)*J71/(Kno+J71)*I71/(Ks_an+I71)*H71
=(p+(N72/eno_max)^n_anox*mumax_an)*Ki/(Ki+O72)*J72/(Kno+J72)*I72/(Ks_an+I72)*H72
=(p+(N73/eno_max)^n_anox*mumax_an)*Ki/(Ki+O73)*J73/(Kno+J73)*I73/(Ks_an+I73)*H73
=(p+(N74/eno_max)^n_anox*mumax_an)*Ki/(Ki+O74)*J74/(Kno+J74)*I74/(Ks_an+I74)*H74
=(p+(N75/eno_max)^n_anox*mumax_an)*Ki/(Ki+O75)*J75/(Kno+J75)*I75/(Ks_an+I75)*H75
```

fxh	fss
=R25+S25-(b+D)*H25	=(-1/Yc_ox)*R25-(1/Yc_an)*S25+(1000-I25)*D
=R26+S26-(b+D)*H26	=(-1/Yc_ox)*R26-(1/Yc_an)*S26+(1000-I26)*D
=R27+S27-(b+D)*H27	=(-1/Yc_ox)*R27-(1/Yc_an)*S27+(1000-I27)*D
=R28+S28-(b+D)*H28	=(-1/Yc_ox)*R28-(1/Yc_an)*S28+(1000-I28)*D
=R29+S29-(b+D)*H29	=(-1/Yc_ox)*R29-(1/Yc_an)*S29+(1000-I29)*D
=R30+S30-(b+D)*H30	=(-1/Yc_ox)*R30-(1/Yc_an)*S30+(1000-I30)*D
=R31+S31-(b+D)*H31	=(-1/Yc_ox)*R31-(1/Yc_an)*S31+(1000-I31)*D
=R32+S32-(b+D)*H32	=(-1/Yc_ox)*R32-(1/Yc_an)*S32+(1000-I32)*D
=R33+S33-(b+D)*H33	=(-1/Yc_ox)*R33-(1/Yc_an)*S33+(1000-I33)*D
=R34+S34-(b+D)*H34	=(-1/Yc_ox)*R34-(1/Yc_an)*S34+(1000-I34)*D
=R35+S35-(b+D)*H35	=(-1/Yc_ox)*R35-(1/Yc_an)*S35+(1000-I35)*D
=R36+S36-(b+D)*H36	=(-1/Yc_ox)*R36-(1/Yc_an)*S36+(1000-I36)*D
=R37+S37-(b+D)*H37	=(-1/Yc_ox)*R37-(1/Yc_an)*S37+(1000-I37)*D
=R38+S38-(b+D)*H38	=(-1/Yc_ox)*R38-(1/Yc_an)*S38+(1000-I38)*D
=R39+S39-(b+D)*H39	=(-1/Yc_ox)*R39-(1/Yc_an)*S39+(1000-I39)*D
=R40+S40-(b+D)*H40	=(-1/Yc_ox)*R40-(1/Yc_an)*S40+(1000-I40)*D
=R41+S41-(b+D)*H41	=(-1/Yc_ox)*R41-(1/Yc_an)*S41+(1000-I41)*D
=R42+S42-(b+D)*H42	=(-1/Yc_ox)*R42-(1/Yc_an)*S42+(1000-I42)*D
=R43+S43-(b+D)*H43	=(-1/Yc_ox)*R43-(1/Yc_an)*S43+(1000-I43)*D
=R44+S44-(b+D)*H44	=(-1/Yc_ox)*R44-(1/Yc_an)*S44+(1000-I44)*D
=R45+S45-(b+D)*H45	=(-1/Yc_ox)*R45-(1/Yc_an)*S45+(1000-I45)*D
=R46+S46-(b+D)*H46	=(-1/Yc_ox)*R46-(1/Yc_an)*S46+(1000-I46)*D
=R47+S47-(b+D)*H47	=(-1/Yc_ox)*R47-(1/Yc_an)*S47+(1000-I47)*D
=R48+S48-(b+D)*H48	=(-1/Yc_ox)*R48-(1/Yc_an)*S48+(1000-I48)*D
=R49+S49-(b+D)*H49	=(-1/Yc_ox)*R49-(1/Yc_an)*S49+(1000-I49)*D
=R50+S50-(b+D)*H50	=(-1/Yc_ox)*R50-(1/Yc_an)*S50+(1000-I50)*D
=R51+S51-(b+D)*H51	=(-1/Yc_ox)*R51-(1/Yc_an)*S51+(1000-I51)*D
=R52+S52-(b+D)*H52	=(-1/Yc_ox)*R52-(1/Yc_an)*S52+(1000-I52)*D
=R53+S53-(b+D)*H53	=(-1/Yc_ox)*R53-(1/Yc_an)*S53+(1000-I53)*D
=R54+S54-(b+D)*H54	=(-1/Yc_ox)*R54-(1/Yc_an)*S54+(1000-I54)*D
=R55+S55-(b+D)*H55	=(-1/Yc_ox)*R55-(1/Yc_an)*S55+(1000-I55)*D
=R56+S56-(b+D)*H56	=(-1/Yc_ox)*R56-(1/Yc_an)*S56+(1000-I56)*D
=R57+S57-(b+D)*H57	=(-1/Yc_ox)*R57-(1/Yc_an)*S57+(1000-I57)*D
=R58+S58-(b+D)*H58	=(-1/Yc_ox)*R58-(1/Yc_an)*S58+(1000-I58)*D
=R59+S59-(b+D)*H59	=(-1/Yc_ox)*R59-(1/Yc_an)*S59+(1000-I59)*D
=R60+S60-(b+D)*H60	=(-1/Yc_ox)*R60-(1/Yc_an)*S60+(1000-I60)*D
=R61+S61-(b+D)*H61	=(-1/Yc_ox)*R61-(1/Yc_an)*S61+(1000-I61)*D
=R62+S62-(b+D)*H62	=(-1/Yc_ox)*R62-(1/Yc_an)*S62+(1000-I62)*D
=R63+S63-(b+D)*H63	=(-1/Yc_ox)*R63-(1/Yc_an)*S63+(1000-I63)*D
=R64+S64-(b+D)*H64	=(-1/Yc_ox)*R64-(1/Yc_an)*S64+(1000-I64)*D
=R65+S65-(b+D)*H65	=(-1/Yc_ox)*R65-(1/Yc_an)*S65+(1000-I65)*D
=R66+S66-(b+D)*H66	=(-1/Yc_ox)*R66-(1/Yc_an)*S66+(1000-I66)*D
=R67+S67-(b+D)*H67	=(-1/Yc_ox)*R67-(1/Yc_an)*S67+(1000-I67)*D
=R68+S68-(b+D)*H68	=(-1/Yc_ox)*R68-(1/Yc_an)*S68+(1000-I68)*D
=R69+S69-(b+D)*H69	=(-1/Yc_ox)*R69-(1/Yc_an)*S69+(1000-I69)*D
=R70+S70-(b+D)*H70	=(-1/Yc_ox)*R70-(1/Yc_an)*S70+(1000-I70)*D
=R71+S71-(b+D)*H71	=(-1/Yc_ox)*R71-(1/Yc_an)*S71+(1000-I71)*D
=R72+S72-(b+D)*H72	=(-1/Yc_ox)*R72-(1/Yc_an)*S72+(1000-I72)*D
=R73+S73-(b+D)*H73	=(-1/Yc_ox)*R73-(1/Yc_an)*S73+(1000-I73)*D
=R74+S74-(b+D)*H74	=(-1/Yc_ox)*R74-(1/Yc_an)*S74+(1000-I74)*D
=R75+S75-(b+D)*H75	=(-1/Yc_ox)*R75-(1/Yc_an)*S75+(1000-I75)*D

fsn	fEo	fEno
=-((1/Yc_an)/(2.86*Yc_an))*S25	=P25-(bO+D)*K25	=Q25-(bNO+D)*L25
=-((1/Yc_an)/(2.86*Yc_an))*S26	=P26-(bO+D)*K26	=Q26-(bNO+D)*L26
=-((1/Yc_an)/(2.86*Yc_an))*S27	=P27-(bO+D)*K27	=Q27-(bNO+D)*L27
=-((1/Yc_an)/(2.86*Yc_an))*S28	=P28-(bO+D)*K28	=Q28-(bNO+D)*L28
=-((1/Yc_an)/(2.86*Yc_an))*S29	=P29-(bO+D)*K29	=Q29-(bNO+D)*L29
=-((1/Yc_an)/(2.86*Yc_an))*S30	=P30-(bO+D)*K30	=Q30-(bNO+D)*L30
=-((1/Yc_an)/(2.86*Yc_an))*S31	=P31-(bO+D)*K31	=Q31-(bNO+D)*L31
=-((1/Yc_an)/(2.86*Yc_an))*S32	=P32-(bO+D)*K32	=Q32-(bNO+D)*L32
=-((1/Yc_an)/(2.86*Yc_an))*S33	=P33-(bO+D)*K33	=Q33-(bNO+D)*L33
=-((1/Yc_an)/(2.86*Yc_an))*S34	=P34-(bO+D)*K34	=Q34-(bNO+D)*L34
=-((1/Yc_an)/(2.86*Yc_an))*S35	=P35-(bO+D)*K35	=Q35-(bNO+D)*L35
=-((1/Yc_an)/(2.86*Yc_an))*S36	=P36-(bO+D)*K36	=Q36-(bNO+D)*L36
=-((1/Yc_an)/(2.86*Yc_an))*S37	=P37-(bO+D)*K37	=Q37-(bNO+D)*L37
=-((1/Yc_an)/(2.86*Yc_an))*S38	=P38-(bO+D)*K38	=Q38-(bNO+D)*L38
=-((1/Yc_an)/(2.86*Yc_an))*S39	=P39-(bO+D)*K39	=Q39-(bNO+D)*L39
=-((1/Yc_an)/(2.86*Yc_an))*S40	=P40-(bO+D)*K40	=Q40-(bNO+D)*L40
=-((1/Yc_an)/(2.86*Yc_an))*S41	=P41-(bO+D)*K41	=Q41-(bNO+D)*L41
=-((1/Yc_an)/(2.86*Yc_an))*S42	=P42-(bO+D)*K42	=Q42-(bNO+D)*L42
=-((1/Yc_an)/(2.86*Yc_an))*S43	=P43-(bO+D)*K43	=Q43-(bNO+D)*L43
=-((1/Yc_an)/(2.86*Yc_an))*S44	=P44-(bO+D)*K44	=Q44-(bNO+D)*L44
=-((1/Yc_an)/(2.86*Yc_an))*S45	=P45-(bO+D)*K45	=Q45-(bNO+D)*L45
=-((1/Yc_an)/(2.86*Yc_an))*S46	=P46-(bO+D)*K46	=Q46-(bNO+D)*L46
=-((1/Yc_an)/(2.86*Yc_an))*S47	=P47-(bO+D)*K47	=Q47-(bNO+D)*L47
=-((1/Yc_an)/(2.86*Yc_an))*S48	=P48-(bO+D)*K48	=Q48-(bNO+D)*L48
=-((1/Yc_an)/(2.86*Yc_an))*S49	=P49-(bO+D)*K49	=Q49-(bNO+D)*L49
=-((1/Yc_an)/(2.86*Yc_an))*S50	=P50-(bO+D)*K50	=Q50-(bNO+D)*L50
=-((1/Yc_an)/(2.86*Yc_an))*S51	=P51-(bO+D)*K51	=Q51-(bNO+D)*L51
=-((1/Yc_an)/(2.86*Yc_an))*S52	=P52-(bO+D)*K52	=Q52-(bNO+D)*L52
=-((1/Yc_an)/(2.86*Yc_an))*S53	=P53-(bO+D)*K53	=Q53-(bNO+D)*L53
=-((1/Yc_an)/(2.86*Yc_an))*S54	=P54-(bO+D)*K54	=Q54-(bNO+D)*L54
=-((1/Yc_an)/(2.86*Yc_an))*S55	=P55-(bO+D)*K55	=Q55-(bNO+D)*L55
=-((1/Yc_an)/(2.86*Yc_an))*S56	=P56-(bO+D)*K56	=Q56-(bNO+D)*L56
=-((1/Yc_an)/(2.86*Yc_an))*S57	=P57-(bO+D)*K57	=Q57-(bNO+D)*L57
=-((1/Yc_an)/(2.86*Yc_an))*S58	=P58-(bO+D)*K58	=Q58-(bNO+D)*L58
=-((1/Yc_an)/(2.86*Yc_an))*S59	=P59-(bO+D)*K59	=Q59-(bNO+D)*L59
=-((1/Yc_an)/(2.86*Yc_an))*S60	=P60-(bO+D)*K60	=Q60-(bNO+D)*L60
=-((1/Yc_an)/(2.86*Yc_an))*S61	=P61-(bO+D)*K61	=Q61-(bNO+D)*L61
=-((1/Yc_an)/(2.86*Yc_an))*S62	=P62-(bO+D)*K62	=Q62-(bNO+D)*L62
=-((1/Yc_an)/(2.86*Yc_an))*S63	=P63-(bO+D)*K63	=Q63-(bNO+D)*L63
=-((1/Yc_an)/(2.86*Yc_an))*S64	=P64-(bO+D)*K64	=Q64-(bNO+D)*L64
=-((1/Yc_an)/(2.86*Yc_an))*S65	=P65-(bO+D)*K65	=Q65-(bNO+D)*L65
=-((1/Yc_an)/(2.86*Yc_an))*S66	=P66-(bO+D)*K66	=Q66-(bNO+D)*L66
=-((1/Yc_an)/(2.86*Yc_an))*S67	=P67-(bO+D)*K67	=Q67-(bNO+D)*L67
=-((1/Yc_an)/(2.86*Yc_an))*S68	=P68-(bO+D)*K68	=Q68-(bNO+D)*L68
=-((1/Yc_an)/(2.86*Yc_an))*S69	=P69-(bO+D)*K69	=Q69-(bNO+D)*L69
=-((1/Yc_an)/(2.86*Yc_an))*S70	=P70-(bO+D)*K70	=Q70-(bNO+D)*L70
=-((1/Yc_an)/(2.86*Yc_an))*S71	=P71-(bO+D)*K71	=Q71-(bNO+D)*L71
=-((1/Yc_an)/(2.86*Yc_an))*S72	=P72-(bO+D)*K72	=Q72-(bNO+D)*L72
=-((1/Yc_an)/(2.86*Yc_an))*S73	=P73-(bO+D)*K73	=Q73-(bNO+D)*L73
=-((1/Yc_an)/(2.86*Yc_an))*S74	=P74-(bO+D)*K74	=Q74-(bNO+D)*L74
=-((1/Yc_an)/(2.86*Yc_an))*S75	=P75-(bO+D)*K75	=Q75-(bNO+D)*L75

APPENDIX B
SEMI-LOGARITHMIC PLOTS

The semi-logarithmic plots of data from the anoxic phase of experiments discussed in Chapter 5 are shown in this section. Growth of cultures using acetate or malate as carbon substrate resulted in two straight lines of different slopes (which represent two different growth rates). Growth of *Paracoccus pantotrophus* using caproate resulted in one straight line with one slope throughout the entire anoxic phase.

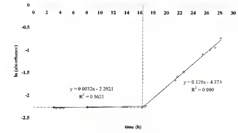


Figure B.1 Anoxic specific growth rate and lag length determination for *Pseudomonas denitrificans* in acetate

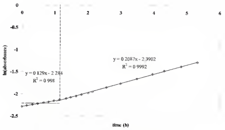


Figure B-2. Anoxic specific growth rate and lag length determination for *Paracoccus pantotrophus* in acetate.

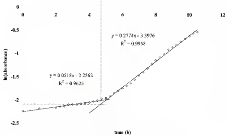


Figure B-3. Anoxic specific growth rate and lag length determination for *Pseudomonas denitrificans* in malate

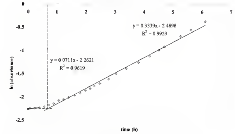


Figure B-4 Anoxic specific growth rate and lag length determination for *Paracoccus pantotrophus* in malate.

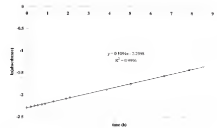


Figure B-5: Anoxic specific growth rate and lag length determination for *Paracoccus pantotrophus* in caproate

APPENDIX C
ADDITIONAL MODEL FITS

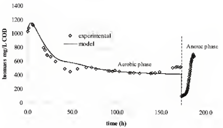


Figure C-1 Preliminary model fit for *Pseudomonas denitrificans* on malate.

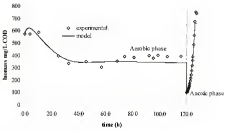


Figure C-2 Preliminary model fit for *Paracoccus pantotrophus* in malate

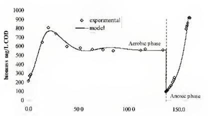


Figure C-3 Preliminary model fit for *Paracoccus pantotrophus* in caproate

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